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Original Research Article

Efficacy of RBC histogram in the diagnosis of morphological types of anaemia compared with peripheral smear

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ABSTRACT

Background: Anaemia constitutes an important diagnostic and clinical category of haematological disorders. Along with peripheral smear histogram is used to interpret the abnormal RBC morphology. The present study is to find out the efficacy of RBC histogram in diagnosis of morphological types of anaemia.
Methods: A total of 354 blood samples of adults >18 years, Hb <10 mg/dl included in the study conducted at Medical College, Kottayam. Peripheral smear evaluated for RBC morphology without referring to the histogram. RBC histogram from same sample analyzed separately. The results obtained, classified into 4 morphological types of anemia -Normocytic normochromic, microcytic hypochromic, macrocytic and hemolytic anemias. Sensitivity, specificity, positive predictive value, negative predictive value and accuracy were calculated.
Results: The major proportion of study is in the age group of 51-70. Major morphologic type in both, is normocytic. Concordant samples for normocytic - 161, microcytic- 97, macrocytic- 7, Hemolytic- 1. Histogram show more sensitivity and less specificity for normocytic. Microcytic has less sensitivity, more specificity. Macrocytic showed >90% sensitivity and specificity. Hemolytic has less sensitivity, more specificity. Overall sensitivity is 75%.
Conclusions: Histogram is efficient in detecting normocytic and microcytic anaemia. In macrocytic anaemia among 40 cases detected by histogram only 7 showed concordance because histogram may detect slight variation in morphology not appreciable on light microscopy. In haemolytic anaemia only one case detected by histogram. The discordance is due to low sample size.

Keywords: Histogram, Peripheral smear, Anaemia

INTRODUCTION

Anaemia constitutes an important diagnostic and clinical category of hematological disorders prevalent all over the world. As of 2010 statistics prevalence of anaemia was approximately 32.9% worldwide resulting 68.36 million years lived with disability.[1] Anaemia is functionally defined as a decrease in the competence of blood to carry oxygen to tissues, thereby causing tissue hypoxia. In clinical medicine, it refers to a decrease in the normal concentration of hemoglobin or erythrocytes.

The RBC histogram is an integral part of automated hematology analysis and is now routinely available on all automated cell counters. The histograms provide major clues in diagnosis and management of significant red cell disorders.

The PBF exposes the morphology of peripheral blood cells, which ensures its place in the morphologic diagnosis of various primary and secondary blood and blood related diseases. Its diagnostic relevance has not

been lessened by advances in haematology automation and molecular techniques.

METHODS

Research question

What is the efficacy of RBC histogram in the diagnosis of morphological types of anaemia of adults more than 18 years, compared to peripheral smear as gold standard?

Diagnostic test evaluation study conducted at Department of Pathology, Govt. Medical College, Kottayam, from March 2020-August 2021.

Sample size

$$N = \frac{Z_{(1-\frac{\propto}{2})^2} \times Sensitivity \times (1 - Sensitivity)}{(deviation)^2}$$

Where $Z_{(1-\frac{\propto}{2})^2} = Constant = (1.96)^2 \approx 4$

According to study done by Choudhary S et al.[2] Overall sensitivity of histogram was 67%, (From my pilot study with 25 samples also, sensitivity was 66.7 ≅ 67%), deviation taken as absolute error as 5, N is calculated as

$$N = \frac{4 \times 67 \times 33}{25} = 353.76 \cong 354$$

Inclusion criteria

All adults more than 18 years with Hb less than 10 gram/dl.

Exclusion criteria

Pregnancy, haematological malignancies, previously diagnosed and treated cases of anaemia.

Study procedure

After obtaining permission for the present study, 354 blood samples of adults more than 18 years of age and Hb level less than 10 mg/dl were included in my study conducted at Govt. Medical College, Kottayam.

Leishman-stained peripheral smear of 2ml EDTA sample were evaluated for RBC morphology to make an impression on the type of anaemia without referring to the histogram. Evaluation was done by faculty of pathology.

RBC histogram from same sample was analysed separately. Evaluation was done by faculty of pathology, different from the above.

Both the group of evaluation were blinded as to the results.

The results obtained from both methods are then classified into 4 morphological types of anaemia – Normocytic normochromic, microcytic hypochromic, macrocytic and haemolytic anaemias.

Sensitivity, specificity, positive predictive value, negative predictive value and accuracy were calculated.

Data management and analysis

The data was entered in Microsoft excel and further statistical analysis was done using SPSS software (version 26).

The statistical methods used were:

Mean, frequency and proportion for age, gender

Morphologic diagnosis of anaemias of 4 types Normocytic normochromic, microcytic hypochromic, macrocytic, hemolytic by histogram and peripheral smear.

Sensitivity, Specificity, positive predictive value, negative predictive value and accuracy of histogram in diagnosing morphological types of anaemia was compared with peripheral smear which is the gold standard.

RESULTS

Among of 354 study samples, 200 were females and 154 were males.

The major proportion of study sample falls in the age group of 51-70. Major morphologic type in both histogram and peripheral smear in this age group is normocytic normochromic among males and females.

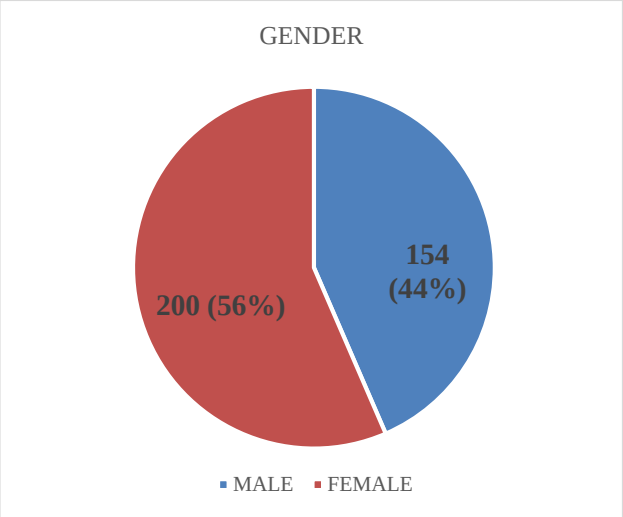

Figure 1: Gender distribution of study samples.

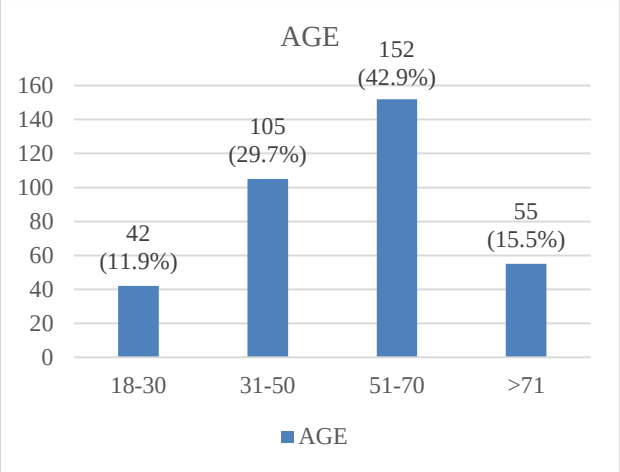

Figure 2: Age distribution of study samples.

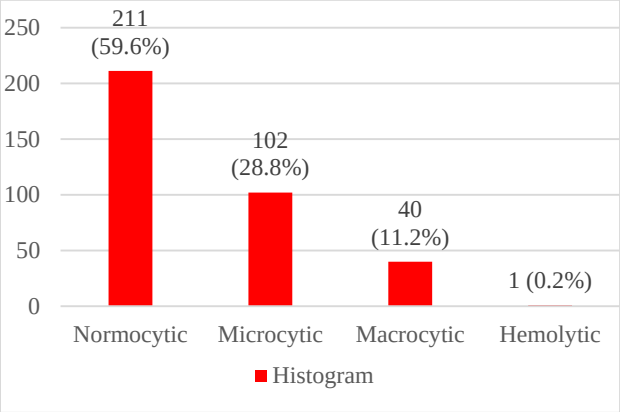

Figure 3: Morphologic diagnosis of anaemia by histogram.

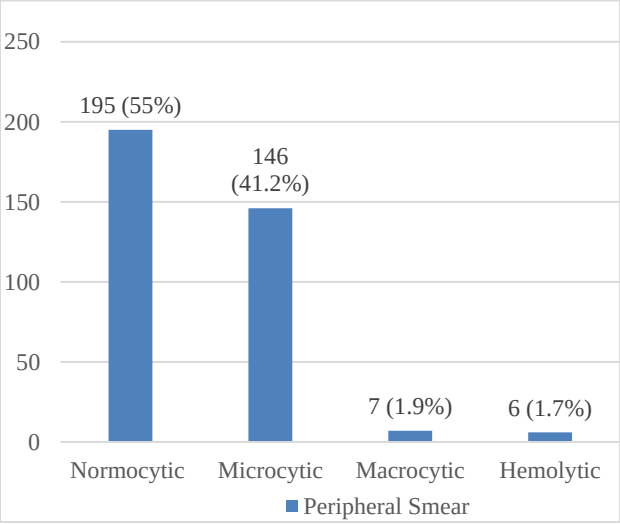

Figure 4: Morphologic diagnosis of anaemia by peripheral smear.

Tables for calculation of diagnostic accuracy of histogram in comparison to peripheral smear

Normocytic normochromic anaemia

Table 1: Normocytic normochromic anaemia table for calculating diagnostic accuracy of histogram in comparison to peripheral smear.

Normocytic	Peripheral smear (positive)	Peripheral smear (negative)
Histogram (positive)	161 (true positive)	50 (false positive)
Histogram (negative)	34 (false negative)	109 (true negative)

From the table, diagnostic accuracy of normocytic normochromic anaemia was calculated as,

$$\text{Sensitivity} = \frac{TP}{(TP+FN)} \times 100 = \frac{161}{195} \times 100 = 82.56\%$$

$$\text{Specificity} = \frac{TN}{(TN+FP)} \times 100 = \frac{109}{159} \times 100 = 68.55\%$$

$$\text{Positive Predictive Value} = \frac{TP}{(TP+FP)} \times 100 = \frac{161}{211} \times 100 = 76.3\%$$

$$\text{Negative Predictive Value} = \frac{TN}{(TN+FN)} \times 100 = \frac{109}{143} \times 100 = 76.2\%$$

$$\text{Accuracy} = \frac{TP+TN}{(TP+FP+TN+FN)} \times 100 = \frac{270}{354} \times 100 = 76.27\%$$

Microcytic hypochromic anaemia

Table 2: Microcytic hypochromic anaemia table for calculating diagnostic accuracy of histogram in comparison to peripheral smear.

Microcytic	Peripheral smear (positive)	Peripheral smear (negative)
Histogram (positive)	97 (true positive)	5 (false positive)
Histogram (negative)	49 (false negative)	203 (true negative)

From the table diagnostic accuracy of microcytic hypochromic anaemia was calculated as,

$$\text{Sensitivity} = \frac{TP}{(TP+FN)} \times 100 = \frac{97}{146} \times 100 = 66.44\%$$

$$\text{Specificity} = \frac{TN}{(TN+FP)} \times 100 = \frac{203}{208} \times 100 = 97.59\%$$

$$\text{Positive Predictive Value} = \frac{TP}{(TP+FP)} \times 100 = \frac{97}{102} \times 100 = 95\%$$

$$\text{Negative Predictive Value} = \frac{TN}{(TN+FN)} \times 100 = \frac{203}{252} \times 100 = 80.50\%$$

Accuracy = $\frac{TP+TN}{(TP+FP+TN+FN)} \times 100 = \frac{300}{354} \times 100 =$ 84.75%

Macrocytic anaemia

Table 3: Macrocytic anaemia table for calculating diagnostic accuracy of histogram in comparison to peripheral smear.

Macrocytic	Peripheral smear (positive)	Peripheral smear (negative)
Histogram (positive)	7 (true positive)	33 (false positive)
Histogram (negative)	0 (false negative)	314 (true negative)

Bone marrow studies were not done.

From the table, diagnostic accuracy of macrocytic anaemia was calculated as,

Sensitivity = $\frac{TP}{(TP+FN)} \times 100 = \frac{7}{7} \times 100 =$ 100%

Specificity = $\frac{TN}{(TN+FP)} \times 100 = \frac{314}{347} \times 100 =$ 90.48%

Positive Predictive Value = $\frac{TP}{(TP+FP)} \times 100 = \frac{7}{40} \times 100 =$ 17.50%

Negative Predictive Value = $\frac{TN}{(TN+FN)} \times 100 = \frac{314}{314} \times 100 =$ 100%

Accuracy = $\frac{TP+TN}{(TP+FP+TN+FN)} \times 100 = \frac{321}{354} \times 100 =$ 90.68%

4. ***Hemolytic anaemia***

Table 4: Hemolytic anaemia table for calculating diagnostic accuracy of histogram in comparison to peripheral smear.

Hemolytic	Peripheral smear (positive)	Peripheral smear (negative)
Histogram (positive)	1 (true positive)	0 (false positive)
Histogram (negative)	5 (false negative)	348 (true negative)

From the table diagnostic accuracy of hemolytic anaemia was calculated as,

Sensitivity = $\frac{TP}{(TP+FN)} \times 100 = \frac{1}{6} \times 100 =$ 16.66%

Specificity = $\frac{TN}{(TN+FP)} \times 100 = \frac{348}{348} \times 100 =$ 100%

Positive Predictive Value = $\frac{TP}{(TP+FP)} \times 100 = \frac{1}{1} \times 100 =$ 100%

Negative Predictive Value = $\frac{TN}{(TN+FN)} \times 100 = \frac{348}{353} \times 100 =$ 98.50%

Accuracy = $\frac{TP+TN}{(TP+FP+TN+FN)} \times 100 = \frac{349}{354} \times 100 =$ 98.59%

The overall sensitivity was calculated using formula, Cases correlated/Total cases multiplied by 100.

For study done by Sarita et al, Overall sensitivity was 67%. In present study it is calculated as 266/354 x100 = 75%.

DISCUSSION

In present study, the age range of the population was between 18 to 100 years, predominant age group is 51-70 years.

The female to male ratio was 1.3:1, Study done by Saritha et al, Rushika et al also had similar findings.[3] In our study both histogram and peripheral smear show female were the predominant population affected with all the 4 morphological types of anaemia, except for normocytic and macrocytic anaemia males are predominant population in older age group.

In our study predominant population is normocytic normochromic both by histogram and peripheral smear and it may be attributed to our population having a good socioeconomic status and proper nutritional awareness also because of our study having excluded pregnant females. Hence, comparing with other study of Dr. Alpesh Goswami. microcytic predominance may be due to study having conducted at northern parts of India where it is reported as having poor socioeconomic status and poor nutritional awareness.[4]

The females in 18-50 age group are in reproductive age group; hence, menstrual cycle related disorders may be a cause for predominance of microcytic morphology seen in our study. Iron deficiency anaemia is the most prevalent cause of microcytic anaemia, which affects mostly women in their reproductive years. Iron deficiency is a significant problem in our nation. The body needs more iron when it grows rapidly and when frequent blood loss occurs (menstruation).[5] Thus, women in reproductive age group are at high risk of developing iron deficiency anemia. Study done by Saritha et al and Anuradha et al had similar findings.[6] The discrepancy of results in categorizing microcytic anemia in Peripheral smear 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 misinterpreted as microcytic RBC by automated cell counter.

In both sexes of age group 51 to more than 70, normocytic normochromic is the predominant morphology by both histogram and peripheral smear because in this age group chronic disorders are the main cause for anaemia.[7] In macrocytic anaemia, a right shift with a broad-based curve indicates a low Hb level and a macrocytic blood image. The causes of macrocytosis range from benign to malignant, and determining the aetiology requires a detailed evaluation. Macrocytosis can strike at any age, although it is more common among the elderly. 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.

In our study, only one hemolytic anaemia is detected by histogram, but peripheral smear detected 6 hemolytic anaemias. RBC histogram in our case of hemolytic anemia showed broad-based curves. This finding is mainly because of the presence of fragmented RBCs which are counted as microcytes, 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. Garg et al. pointed out that the broad-based histograms with right skewing and elevated RDW with low hematocrit value points toward hemolytic anaemia.[8]

Table 5: Comparison of sensitivity and specificity of various morphological types with study done by Sarita et al.

	Sensitivity		Specificity	
	Sarita et al, 2018 Parent study	Present study, 2020	Sarita et al, 2018 Parent study	Present study, 2020
Normocytic	47.80	82.56	97.30	68.55
Microcytic	93.90	66.44	64.20	97.59
Macrocytic	93.30	100	98.70	90.48
Hemolytic		16.66		100

Table 6: Comparison of distribution of concordant and discordant cases with similar studies.

	Total	Concordant	Discordant
Present study, 2020	354	266 (75.10%)	88 (24.90%)
Sarita et al, 2018 Parent study	600	402 (67%)	198 (33%)
Farah E et al, 2013	350	274 (78.30%)	76 (21.70%)
Radadiya P et al, 2015	100	72 (72%)	28 (28%)

Our study shows that histogram is more sensitive in detecting normocytic normochromic anaemia than in study done by Dr. Sarita et al, but the specificity is less. In case of Microcytic hypochromic anaemia sensitivity is less than the parent study but specificity is more. The discordance is due to, our study was done in teritiary care center, where selected cases are evaluated, our predominant study population is 51-70 years and we excluded paediatric and pregnant cases from our cross section. But the study done by Dr. Sarita et al was done in rural population and cross section included all age groups.

For study done by Sarita et al, Overall sensitivity was 67%. In present study it is calculated as 75%. Our study showed a good overall sensitivity for histogram in detecting anaemia. Hence, we can conclude that the histogram may be used as a screening tool that can minimize the use of peripheral smear examinations, and by comparing the results of both approaches, we can diagnose the majority of anaemia cases, similar finding was observed in study done by Sasidhar et al.[9]

Concordant typing was present among 266 cases (75.1%) and discordance was present among 88 (24.9%) cases. In concordant cases, 161 were normocytic, 97 were microcytic, 7 were macrocytic and 1 was hemolytic. Discordance can be due to presence of agglutinated RBCs, fragmented RBCs or abnormal blood cells which were not detected by automated analyzer. Our concordance is similar to study done by Farah E et al and Radadiya et al.[10]

Florence Aslina et al found that peripheral blood smear was more sensitive than RBC indices for identifying early microcytic changes because the MCV represented the mean of the distribution curve and was insensitive to the presence of small numbers of macrocytes. This present study also shows similar findings.[12]

Study done by Benie T Constantiano stated that, from reviewing the histograms, one can get a good idea of what to expect when actually evaluating the peripheral blood film. Unfortunately, most technologists have a limited understanding in correlating the graphic displays

with the morphological findings. This is probably because graphical representation of results, such as scatter plots or histograms, have been largely ignored in favor of the RDW, hemoglobin distribution width, and reticulocyte hemoglobin content that provide very useful information along with the red cell indices, that have been traditionally used.[13]

The discrepancy between automation and manual scan of peripheral blood in the measurement of haemoglobin and red blood cell count can result in misclassification for the diagnosis of anaemia. This signifies that manual microscopic method has an advantage over the automated method. In present study histogram was more sensitive for the diagnosis of normocytic and macrocytic anaemia and was more specific for microcytic, macrocytic, and hemolytic anaemia.

Peripheral blood films can differentiate not only morphological types of anaemia but also hemoglobinopathies and other blood disorders. Other conditions like presence of platelet clumps, platelet satellitism and giant platelets could be identified by peripheral smear examination only. Hence the diagnosis if anaemias peripheral blood film may be considered as gold standard.

Limitations of study

Peripheral smear slides get fade over time, so proper storage was not possible so we digitalized the data.

CONCLUSION

In the present study, the overall sensitivity of histogram in determining anaemia is 75%. Histogram is efficient in detecting normocytic normochromic and microcytic hypochromic anaemia. Among the 211 cases detected as normocytic normochromic by histogram 161 cases show concordance with peripheral smear and 102 cases detected as microcytic by histogram 97 cases show concordance with peripheral smear. In case of macrocytic anaemia among 40 cases detected by histogram only 7 cases show concordance with peripheral blood film. Thus, histogram may be more efficient in detecting macrocytic anaemia because it may detect slight variation in morphology which may not be appreciable on light microscopy. In case of haemolytic anaemia only 1 case was detected by histogram. The discordance could be due to low sample size.

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