Indian Journal of Basic and Applied Medical Research; December 2018: Vol.-8, Issue- 1, P. 135 - 141

Original Article

Sensitivity of Red Cell Histogram and CBC parameters against Peripheral Blood Smear in Various Anemias

[1] Dr Sarita Choudhary,[2] Dr Sandhya Bordia , [3]Dr Kavita Choudhary

[1]Senior Demonstrator, Department of Pathology, Pandit Deendayal Upadhyaya Medical College, Churu, Rajasthan, India

[2] Professor, Department of Pathology, RNT Medical College, Udaipur, Rajasthan, India

[3] Assistant Professor, Department of Community Medicine, Pandit Deendayal Upadhyaya Medical College, Churu, Rajasthan, India

Corresponding author: Dr Kavita Choudhary

Abstract:

Background: Now a days; automated peripheral blood count for the diagnosis of anemia is widely accepted in routine practice and the instrument is also quite sophisticated but there is need to depend on manual microscopic scan of peripheral smear for the diagnosis and treatment of different types of anemias. The correlation between automated haematology analyzer and manual microscopic scan is rare and conflicting. Hence the present study was planned to evaluate the sensitivity of red cell histogram against peripheral smear.

Objective: To evaluate the sensitivity of Red Cell Histogram against the Gold Standard (Peripheral Blood Film).

Methodology: It was a cross sectional study and conducted at Central Diagnostic Laboratory at RNT medical College, Udaipur. Blood sample of 600 anemic patients were selected using systematic random sampling and data were analysed using Epi-info 7.0 software by proportion, mean, sensitivity and specificity

Results: All over Sensitivity of histogram was 67%. Sensitivity of different morphological types of anemia was also calculated. Microcytic hypochromic anemia with histogram and RBC indices showed a high sensitivity (93.9%) and lower specificity of 64.2%. Normocytic anemia with histogram and RBC indices showed a high specificity (97.3%) but lower sensitivity of 47.8%. Macrocytic anemia with histogram and RBC indices showed high specificity of 98.7% and sensitivity of 93.3%. Dimorphic anemia with histogram and RBC indices showed high specificity of 95.6% but lower sensitivity of 51%.

Conclusion: Automated hematology analyzer are good for screening purpose but to diagnose and differentiate different types of anemias manual scan with peripheral smear should be method of choice.

Key Words: Red cell Histogram, Peripheral Blood Film, Sensitivity, Specificity

Introduction:

Anemia can be classified by both qualitative and quantitative methods. An initial morphological classification of anemia with integration of red blood cell indices and morphologic characteristic is very useful. (1) Peripheral blood examination has been a window for haematological ongoings since several decades and facilitated interpretation of various haematological disorders and has been a major diagnostic tool especially for etiopathological work up of anemias. Histogram along with absolute counts gives valuable information about the abnormality of sample and need for follow up peripheral blood examination. Various shapes of histogram give hint about the pathology before the blood smear could be examined. Histogram is helpful in comparing the size of patient's cell with normal population. Shift in histogram in one or other direction can be of diagnostic importance. (2) The classic Price Jones

studies on red blood cell diameter heterogeneity quantifies anisocytosis as a coefficient of variation of red blood cell size in a Price Jones curve, which is used to determine the presence and degree of red cell volume heterogeneity.(3,4) Heterogeneity generally identified by peripheral blood film; however recently designed automated particle counters have allowed rapid and precise quantification of volume heterogeneity. (5,6) Special diagnosis of leukemia and other WBC disorders are possible only with peripheral blood film examination. (7) Histogram becomes a practical tool in initial stage of morphological analysis if combined with knowledge of CBC parameters such as red cell indices and RDW. To make the histogram more meaningful it should be compared with a reference normal curve or confirmed by microscopy. (8) Now a days; automated peripheral blood count for the diagnosis of anemia is widely accepted in routine practice and the instrument is also quite sophisticated but there is need to depend on manual microscopic scan of peripheral smear for the diagnosis and treatment of different types of anemias. The correlation between automated haematology analyzer and manual microscopic scan is rare and conflicting. Hence the present study was planned to evaluate the sensitivity of red cell histogram against peripheral smear.

Objective:

To evaluate the sensitivity of Red Cell Histogram against the Gold Standard (Peripheral Blood Film).

Material and Methods:

It was a cross sectional study and conducted at Central Diagnostic Laboratory at RNT medical College, Udaipur. Three ml of EDTA blood sample was collected from anemic patient and histogram was obtained after through mixing. The automated analyzer used in this hospital SYSMEX XS-1000i that is a 5 part differential automated analyzer used for study. Study group was selected by observing Hb% obtained from automated analyzer with respect to age and sex. A simultaneous peripheral smear was also prepared according to standard operating procedure and stained by Giemsa stain. Blood sample of 600 anemic (Hb<10) patients were selected using systematic random sampling and data were analysed using Epi-info 7.0 software by proportion, mean, sensitivity and specificity. The results were considered concordant if typing done by both methods indicated the same morphological type of anemia, otherwise results were considered discordant.

Results:

Table 1: Distribution of cases as per type of Anemia by Histogram &RBC indices

Type of Anemia	Frequency
Normocytic	99(16.5%)
Microcytic	424(70.67%)
Macrocytic	26(4.3%)
Dimorphic	50 (8.3%)
Pancytopenia	27 (4.5%)
Red Cell Agglutinins (cold)	1 (0.2%)
Hemolytic	0 (0.0%)
Thalassemia	1 (0.2%)
Total	600 (100%)

www.ijbamr.com P ISSN: 2250-284X , E ISSN : 2250-2858

The table 1 shows the distribution of cases as per type of Anemia by Histogram &RBC indices. Maximum cases were of microcytic anemia. Only 1 case of red cell agglutinin was found by its characteristic histogram pattern by automated analyzer.

Table 2: Distribution of cases as per Types of Anemia in PBF

Type of Anemia	Frequency (%)
Normocytic	184(30.67%)
Microcytic	316(52.67%)
Macrocytic	20(3.3%)
Dimorphic	49 (8.1%)
Pancytopenia	21 (3.6%)
Red Cell Agglutinins (cold)	2 (0.3%)
Hemolytic	28 (4.7%)
Thalassemia	1 (0.2%)
Total	600 (100%)

Table 2 shows the distribution of type of anemia according to PBF. Maximum cases were of microcytic anemia and 2 cases of red cell agglutinins were found.

Table 3: Comparison between Automated and Manual Morphological typing (PBF) of Anemia

Concordant typing	Discordant typing	Total
402	198	600

Table 3 shows concordant and discordant typing of anemia. 198 cases showed discordant typing, which need to be typed correctly with peripheral smear examination.

Overall Sensitivity of Red cell Histogram = cases correlated / total cases X 100= 402/600x100=67%

Table 4: Sensitivity and Specificity of Histogram with RBC indices in diagnosing microcytic hypochromic anemia against PBF (Gold Standard)

Histogram with RBC indices	PBF		Total
	Microcytic hypochromic anemia present	Microcytic hypochromic anemia absent	
Microcytic hypochromic anemia present	293	103	396
Microcytic hypochromic anemia absent	19	185	204
Total	312	288	600

www.ijbamr.com P ISSN: 2250-284X , E ISSN : 2250-2858

Sensitivity= True positive /Total positive x 100= 293/312x100=93.9%

Specificity= True negative/ Total Negative x 100= 185/288x100=64.2%

Morphological typing of microcytic hypochromic anemia with histogram and RBC indices showed a high sensitivity (93.9%) and specificity of 64.2%.

Table 5: Sensitivity and Specificity of Histogram with RBC indices in diagnosing Normocytic anemia against PBF (Gold Standard)

Histogram with RBC indices	PBF		Total
	Normocytic anemia present	Normocytic anemia absent	
Normocytic anemia present	88	11	99
Normocytic anemia absent	96	405	501
Total	184	416	600

Sensitivity= True positive /Total positive x 100= 88/184x100=47.8%

Specificity= True negative/ Total Negative x 100= 405/416x100=97.3%

Morphological typing of normocytic anemia with histogram and RBC indices showed a high specificity (97.3%) but lower sensitivity of 47.8%.

Table 6: Sensitivity and Specificity of Histogram with RBC indices in diagnosing Macrocytic anemia against PBF (Gold Standard)

Histogram with RBC indices	PBF		Total
	Macrocytic anemia present	Macrocytic anemia absent	
Macrocytic anemia present	19	7	26
Macrocytic anemia absent	1	573	574
Total	20	580	600

Sensitivity= True positive /Total positive x 100= 19/20x100=95%

Specificity= True negative/ Total Negative x 100= 98.7%

Morphological typing of macrocytic anemia with histogram and RBC indices showed high specificity of 98.7% and sensitivity of 93.3%.

www.ijbamr.com P ISSN: 2250-284X , E ISSN : 2250-2858

Table 7: Sensitivity and Specificity of Histogram with RBC indices in diagnosing Dimorphic anemia against PBF (Gold Standard)

Histogram with RBC indices	PBF		Total
	Dimorphic anemia present	Dimorphic anemia absent	
Dimorphic anemia present	25	25	50
Dimorphic anemia absent	24	526	550
Total	49	551	600

Sensitivity= True positive /Total positive x 100= 25/49x100=51%

Specificity= True negative/ Total Negative x 100= 526/551x100=95.6%

Morphological typing of dimorphic anemia with histogram and RBC indices showed high specificity of 95.6% but lower sensitivity of 51%.

Table8: Comparison of platelet counts between Analyser and PBF

Platelets Counts	Frequency in Analyser (%)	Frequency in PBF (%)	χ^2 =16.83
<100 (Reduced)	106 (17.7%)	79 (13.2%)	Df=2
100-400 (Adequate)	416 (69.3%)	476 (79.3%)	
>400 (Thrombocytosis)	78 (13%)	45 (7.5%)	P=0.0001**
Total	600	600	

** Statistically significant

Statistically significant difference was found in platelet counts between analyser and PBF as the p value is <0.05.

Discussion:

In present study the age range of the population was between 5 days to 75 years and the male to female ratio was 0.95:1. In present study histogram and peripheral smear gave different types of results. Concordant typing was present among 402 cases and discordance was present among 198 cases. Discordance can be due to presence of agglutinated RBCs, fragmented RBCs or abnormal blood cells which were not detected by automated analyzer. In present study the histogram of iron deficiency anemia and beta thalassemia trait were shifted to left and the percentage of microcytosis was increased. Although the histogram were similar for both, the degree of anisocytosis, as measured by RDW; differentiates them. We found that patients with macrocytic anemia showed a single small peak on the left of the macrocytic cell because of fragmented RBCs and very small cells. In alcoholic liver disease only single microcytic peak was observed. Abnormal histogram in cold agglutination was due to high titer cold agglutinin causing red cells to agglutinate and interfere with their sizing and enumeration with high MCV. It was

www.ijbamr.com P ISSN: 2250-284X , E ISSN : 2250-2858

corrected by smear examination. In cases of beta thalassemia major; high frequency of small cells was seen at the begning of the histogram. This was due to small particles seen in this disorder, such as red cell fragments, nucleated RBCs, microspherocytes and microcytic cells; which produces an erroneous mean cell volume for the intact cell population.

Table 9: Comparison of concordant and discordant cases in different studies

	Total (n)	Concordant	Discordant
Farah E et al,2013 (9)	350	274(78.3%)	76(21.7%)
Radadiya P et al, 2015 (10)	100	72 (72%)	28(28%)
Present study	600	402(67%)	198(33%)

Pierre and Novis et al reported that automated haematology analyzer were more accurate in detection of morphological abnormalities than the manual count by peripheral blood film.(11) This was in contrast with our finding. 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.(12) This finding was similar to present study. Similarly Lantis et al also supported our study. (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 anemia. This signified that manual microscopic method has advantage over the automated method. (14) In present study histogram was more sensitive for the diagnosis of microcytic and macrocytic anemia and was more specific for normocytic and dimorphic anemia. Statistically significant difference was found in platelet counts between analyser and PBF. This could be due to the fact that hematology analyzar identifies the platelets due to their size and other conditions like red cell fragments and microspherocytes can mimic platelets. Other conditions like presence of platelet clumps, platelet satellitism and giant platelets could be identified by peripheral smear examination only. Peripheral blood films can differentiate not only morphological types of anemia but also hemoglobinopathies and other blood disorders. The validation technique of automated hematology analyzer with peripheral smear should be implemented in each lab to assure performance of test for the maximum benefit of patients.

Conclusion:

The automated hematology analyzers are reliable for the diagnosis of anemia and can be used for screening purpose but to diagnose and differentiate different types of anemia manual scan with peripheral smear should be method of choice.

References:

1. Perkins and Sherrie (1995). Practical diagnosis of hematologic disorders. Chicago, ASCP press.
2. Mary Louise turgeon Clinical Haematology Theory and procedures -4th edition page no 503-506.
3. Price-Jones C. The variation in sizes of red cells. Brit Med J. 1910;2:1418.
4. Price-Jones C. Red Blood Cell Diameters. London: Oxford University Press; 1933.

5. Rowan RM, Fraser C, Gray JH, McDonald GA. The Coulter Counter Model S-Plus–the shape of things to come. Clin Lab Haematol. 1977;1:29-40.
6. Cornbleet PJ, Beuchel J. Red cell distribution width on the Coulter Model S-Plus. Am J Med Tech. 1983;49:865-867.
7. Aroon Kamath M, M.D. Automated blood-cell analyzers. Can you count on them to countwell?. Doctor Lounge Website. Available at: http://www.doctorslounge.com/index.php/blogs/page/17172. Accessed September 13 2016
8. Constantino BT. The red cell histogram and the dimorphic red cell population. LabMed. 2011;42:300-308.
9. Farah E,Mehwish A,Nafisa HA. Comparative Study in the Diagnosis of Anemia by SYSMEX KX-21N hematology analyzer with Peripheral Blood Smear. International Journal of endorsing health science research /vol1/issue2/December,2013.
10. Dr. Radadiya P, Dr.Nandita Mehta, Dr.Hansa Goswami, Dr.R.N.Gonsai. Automated red blood cell analysis compared with routine red blood cell morphology by smear review. NHL Journal of Medical Sciences/ Vol 4/ issue 1; pg 53-57/ Jan 2015.
11. Pierre, R.V. (2002). The demise of the eye count leucocyte differential. Clin Lab Med 22(1), 279-297.
12. Aslinia F, Mazza JJ, Yale SH. Megaloblastic anemia and other causes of macrocytosis. Clin Med Res 2006;4:236-41.
13. Lantis, K. L., Harris, R. J., Davis, G., Renner, N., & Finn, W. G. (2003). Elimination of Instrument-Driven Reflex Manual Differential Leukocyte Counts Optimization of Manual Blood Smear Review Criteria in a High-Volume Automated Hematology Laboratory. American journal of clinical pathology, 119(5), 656-662.
14. Gulati GL, Hyun BH: The automated CBC.A current perspective. Hematol Oncolcin North Am.1994; 8; 593-603.