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Original Article

A Comparative Study of Red Cell Histogram along with CBC parameters and Peripheral Blood Smear in Various Anemias

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Abstract:

Background: Visual scanning of histogram gives a good sense of range, size, shape and other salient features of red cell morphology. Automated haematology analyzers are already an integral part of routine purpose diagnostic laboratory setup. So, we planned a study to evaluate utility of automated RBC parameter in evaluation and diagnosis of anaemia.

Objectives: (1) to interpret the RBC histogram in anemic patients (2) To compare automated histogram patterns and CBC parameters with morphological feature seen on peripheral smear examination with special reference to RDW.

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 and Pearson's correlation.

Results- Out of 600 cases, 402 cases showed concordant typing of anemia with automated analyzer and using peripheral smear examination. 198 cases showed discordant typing, which typed correctly with peripheral smear examination. MCV and RDW were positively moderately correlated with each other in the whole sample and also in Iron deficiency as well as in megaloblastic anemia. MCV and RDW were positively and mildly correlated in mixed deficiency and in anemia of chronic disease. In a similar way MCH and RDW were positively and mildly correlated in all the etiological groups of anemia. Other haematological parameters like Hb, HCT, RBC count and Platelet counts were negatively correlated with RDW. All these correlations were statistically significant.

Conclusion- Though automated analyzer reduces overall workload by its advances of graphical representation, it should be confirmed by microscopy.

Key words: Histogram, CBC, RDW, Peripheral Blood Film

Introduction:

RBC histogram is a graphic representation of particle size distribution and routinely generated by automated cell analyzers as a standard part of automated CBC analysis. Along with CBC parameters (RDW and MCV), histogram has been found abnormal in various haematological conditions and may provide major clues in the diagnosis of significant red cell disorders. (1, 2) Histogram is used along with peripheral blood smear as an aid in monitoring and interpreting abnormal morphological changes. (3, 4) With the help of histogram one can visualize and interpret empirical data that displays morphological changes graphically as points, peaks or valleys or as a line of frequency curve. It helps in visual comparison of the centre and spread of data and data containing two or more variables;

leading to quick and cost effective decision making. (5,6,7,8,9,10,11,12,13,14,15) Variation or degree of anisocytosis in red cell volume is most commonly reported by RDW. The impedance and flowcytometric counters provide this parameter, which is directly calculated from RBC histogram. (16,17,18) The present study intends to compare automated histogram patterns and CBC parameters with morphological features seen on peripheral smear examination; so that the laboratory personnel and clinicians can use this with sufficient accuracy to presumptively diagnose morphological classes of anaemia directly from the automated haematology cell counter forms and correlate with morphological features of peripheral smear examination.

Objectives:

(1) to interpret the RBC histogram in anemic patients (2) To compare automated histogram patterns and CBC parameters with morphological feature seen on peripheral smear examination with special reference to RDW.

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, Chi-Square test. The results were considered concordant if typing done by both methods indicated the same morphological type of anemia, otherwise results were considered discordant. Severity of Anemia was taken as mild if Hb>9 gm%, Moderate if Hb between 7-9 gm% and severe if Hb< 7 gm% (WHO)

Results:

In present study samples of males were 48.7% and samples of females were 51.3%. The age range in the study group of anemia was 5 days to 75 years with 193(32.3%) patient being in the age group 31-45 years followed by 190(31.7%) in age group 16-30 years. The majority of cases in the study group were belong to moderate degree of anemia with frequency of 294(49%) followed by severe degree of anemia with frequency of 184(30.7%). Mean Hb level of study population: 7.6±1.92. Table 1 shows the different variants of histogram in different types of anemia.

Table 1: Histogram variation in different anemias

Types of Anemia	Normal curve	Left shift	Right Shift	Broad base	Bimodal
Normocytic (n=99)	26	3	1	55	0
Microcytic (n=424)	76	411	0	375	3
Macrocytic (n=26)	5	0	22	17	0
Dimorphic (n=50)	0	36	2	49	13
Pancytopenia (n=27)	5	7	21	23	0

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Table 2: Comparison of cases between automation and PBF

Type of Anemia	Frequency (%)	
	Histogram & RBC indices	PBF
Normocytic	99(16.5%)	184(30.67%)
Microcytic	424(70.67%)	316(52.67%)
Macrocytic	26(4.3%)	20(3.3%)
Dimorphic	50 (8.3%)	49 (8.1%)
Pancytopenia	27 (4.5%)	21 (3.6%)
Red Cell Agglutinins (cold)	1 (0.2%)	2 (0.3%)
Hemolytic	0 (0.0%)	28 (4.7%)
Thalassemia	1 (0.2%)	1 (0.2%)
Total	600 (100%)	600 (100%)

Table 2 is a comparative table which shows the overall difference in diagnosing anemias by automation and PBF. Maximum difference was seen in normocytic anemia followed by microcytic anemia.

Table 3: Morphological classification of Anemia based on MCV+ MCHC and MCV+MCH in different etiological groups

Morphological class with MCV+ MCHC and MCV+MCH	Different Etiological groups				
	Iron Deficiency Anemia (n=359)	Megaloblastic Anemia (n=26)	Mixed deficiency Anemia (n=50)	Anemia of Chronic disease (n=46)	Total
Microcytic Hypochromic anemia	248		25	15	288
	357		34	28	419
Microcytic Normochromic anemia	109		10	15	134
			1	2	3
Macrocytic Normochromic anemia		20	1		21
		24	2		26
Macrocytic Hypochromic anemia		4			4
			1		1
Normocytic Normochromic anemia		1	5	16	22
	1	2	10	16	29

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Normocytic	2	1	8		11
Hypochromic anemia	1		3		4
Total	359	26	50	46	481

Table 3 shows the etiologically classifying anemia and their distribution according to morphological class with MCV+ MCHC and MCV+MCH. There were total 435 cases of nutritional anemia (90.4%) with iron deficiency in 359 cases, megaloblastic anemia in 26 cases and mixed deficiency anemia in 50 cases. The anemia of chronic disease was noted in 46 cases (9.56%).

Table 4: Correlation of RDW-SD with other haematological parameters in different etiological groups of Anemia

Parameters	Whole	Iron Deficiency anemia	Megaloblastic anemia	Mixed deficiency anemia	Anemia of Chronic disease
Hb	-0.31(-)	-0.30(-)	-0.31(-)	-0.30(-)	-0.30(-)
HCT	-0.27(-)	-0.26(-)	-0.27(-)	-0.26(-)	-0.26(-)
RBC count	-0.402(- -)	-0.39(-)	-0.40(- -)	-0.39(-)	-0.39(-)
MCV	0.407(++)	0.40(++)	0.40(++)	0.39(+)	0.39(+)
MCH	0.14 (+)	0.14(+)	0.14(+)	0.13(+)	0.13(+)
MCHC	-0.04(NS)	-0.04(NS)	-0.04(NS)	-0.05(NS)	-0.04(NS)
WBC count	0.032(NS)	0.0008(NS)	0.03(NS)	-0.004(NS)	-0.002(NS)
Platelets count	-0.23(-)	-0.23(-)	-0.23(-)	-0.22(-)	-0.23(-)

(++)= Positive Moderate Correlation (Statistically Significant) (Pearson's Correlation Coefficient)

(+)=Positive Mild Correlation (Statistically Significant)

(- -)= Negative Moderate Correlation (Statistically Significant)

(-)=Negative Mild Correlation (Statistically Significant)

NS= Not Significant

Table 4 shows the correlation of RDW with other haematological parameters in different etiological groups of anemia. Pearson's Correlation Coefficient was calculated to see the correlation. MCV and RDW were positively moderately correlated with each other in the whole sample and also in Iron deficiency as well as in megaloblastic anemia. MCV and RDW were positively and mildly correlated in mixed deficiency and in anemia of chronic disease. In a similar way MCH and RDW were positively and mildly correlated in all the etiological groups of anemia. Other haematological parameters like Hb, HCT, RBC count and Platelet counts were negatively correlated with RDW. All these correlations were statistically significant. The remaining other two parameters (WBC count and MCHC) were statistically not correlated with RDW.

Table 5: Distribution of cases with discordant typing

No. of discordant cases	Subdivision	Histogram & red cell indices interpretation	PBF Interpretation
35	21	Microcytic Hypochromic anemia	Dimorphic anemia
	2	Normocytic Hypochromic anemia	
	1	Normocytic Normochromic anemia	
	11	Dimorphic anemia	
28	16	Microcytic Hypochromic anemia	Hemolytic anemia
	1	Microcytic Normochromic anemia	
	1	Normocytic Hypochromic anemia	
	6	Normocytic Normochromic anemia	
	1	Macrocytic Normochromic anemia	
	3	Dimorphic anemia	
2	1	Macrocytic Hypochromic anemia	Macrocytic Normochromic anemia
	1	Dimorphic anemia	
26	4	Normocytic Hypochromic anemia	Microcytic Hypochromic anemia
	4	Normocytic Normochromic anemia	
	17	Dimorphic anemia	
64	26	Microcytic Hypochromic anemia	Normocytic Normochromic anemia
	20	Microcytic Normochromic anemia	
	11	Normocytic Hypochromic anemia	
	4	Macrocytic Normochromic anemia	
	2	Dimorphic anemia	
42	35	Microcytic Hypochromic anemia	Normocytic Hypochromic anemia
	1	Microcytic Normochromic anemia	
	1	Normocytic Hypochromic anemia	
	3	Normocytic Normochromic anemia	
	1	Macrocytic Hypochromic anemia	
	1	Macrocytic Normochromic anemia	
1	1	Microcytic Hypochromic anemia	Red Cell Agglutinin
Total	**198**		

Table 5 shows the distribution of discordant typing. A total 198 samples were found discordant.

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Discussion:

In present study slight female preponderance was found and this finding was similar to other studies. (20) We found maximum patients were in young age group and similar finding was observed by Sandhya I et al in their study. (20) Jadhav M V et al and Sandhya I et al found that maximum cases of their studies were categorized into severe anemia followed by moderate anemia while in present study maximum cases were categorized into moderate anemia followed by severe anemia.(21,20) We found maximum cases of microcytic anemia in present study and the similar finding was obtained by Jitendra C and Sandhya I et al in their studied. (23,20) We found broad base histogram more common in our study which involved cases with normal histogram, left shift, right shift, bimodal and multiple peaks. This finding was also similar with Jitendra C and Sandhya I et al. (23,20) The only difference was that in our study we found overlapping in different types of histograms. In our study RDW-CV gave more accurate results for the diagnosis of microcytic anemia and RDW-SD gave more accurate results for the diagnosis of macrocytic anemia. The similar findings were observed by Caporal A et al. (19) In present study PBF provided additional information in 33% of cases and this finding was similar with Radadiya P et al.(22) Peripheral blood films scan has the ability to identify clinically important cell types such as pencil cell, burr cells, tear drop cells, target cells, sickle cells, blast cells and schistocytes that cannot be quantifiable by the instruments; rather produce flags on the automated results.

Conclusion:

Histogram is a practical working tool in the initial stage of morphological analysis if used with CBC parameters such as RDW and red cell indices. Though automated analyzer reduces overall workload by its advances of graphical representation; it should be confirmed by microscopy.

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