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Correlation of peripheral smear with RBC indices and RBC histograms in the diagnosis of anemia

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Abstract

Introduction: RBC histogram with erythrocyte indices provide clue to the diagnosis of anemia .Along with Peripheral smear it is used to interpret the cause of anemia.

Aim: To correlate peripheral smear findings with RBC histogram patterns and RBCs indices to provide a better approach in the diagnosis of anemia and analyse their limitations.

Materials and Method: 500 anemic patients with haemoglobin levels of less than 11.5gm% on EDTA sample by Beckman coulter LH-780 were included in the present study, leukemia being excluded. An impression was made by examining Leishman stained peripheral smear without referring to the histogram. The RBC histogram and the RBC indices were analysed and the anemia categorised. The correlation between the diagnosis made by Peripheral smear Vs RBC histogram, indices was analysed using Cramer's V (0.523) and Kappa statistics for agreement between the two methods.

Results: The 500 cases included 21.8%, 46%, 8.8%, 17.6% and 5.8% cases of normocytic normochromic, microcytic hypochromic, macrocytic, dimorphic and haemolytic anemia respectively by peripheral smear examination. Analysis by erythrocyte indices and histogram showed similar findings except in macrocytic, dimorphic and hemolytic anemia which were 15.6%, 15.8% and 0.8% respectively. The correlation between the diagnosis made by the two methods was statistically significant, $p<0.0001$ and the agreement between the two methods was moderate (0.518)**.**

Conclusion: RBC histogram and indices should be interpreted in the light of peripheral smear which is indispensable in hemolytic and dimorphic anemia. On the contrary, histogram gives information about subtle changes well in advance of numerical parameters and subjective evaluation.

Keywords: Histogram, Erythrocyte Indices, Peripheral Smear, Anemia, Hemolytic Anemia.

Introduction

Anemia continues to be a major public health problem worldwide, particularly among females of reproductive age in developing countries. Between 1993-2003 World Health Organization's global estimates of anemia prevalence averaged 49%.[(1)] Anemia constitutes an important diagnostic and clinical category of haematological disorders prevalent all over the world. RBC histogram is an integral part of automated analysis which in association with RBC indices, hematocrit and RBC distribution width provides major clues in the diagnosis and management of red cell disorders.[(2)] It provides valuable information not apparent in the numerical data as in megaloblastic anaemia with developing iron deficiency. The small population of microcytic hypochromic cells is identified in the histogram as a double peak[(2)] while MCV being an average value does not reflect the heterogeneity of the RBC population. On the other hand, a double peak histogram curve only indicates the presence of dual cell population which needs to be further correlated with peripheral smear findings. There have been few studies on the utility and comparison of peripheral smear findings with RBC histograms and RBC indices. Thus the present study was undertaken to correlate these in providing a better approach to the diagnosis of anemia.

Materials and Method

500 patients with anemia as determined by decreased haemoglobin levels of less than 11.5gm% on EDTA sample by the 7 part differential autoanalyzer Beckman coulter LH-780 at a Tertiary care hospital from August 2014 to July 2015 were included in the present study. Patients with leukemia were excluded from the study. The present study was a double blind study. Leishman stained peripheral smear of patients were evaluated for RBC morphology making an impression on the type of anemia without referring to the histogram. The RBC histogram and the RBC indices – PCV MCV, MCHC, RDW-CV/RDW-SD were analysed. The position (normal, left shift, right shift) and the shape of RBC histogram (normal bell shaped or Gaussian, widened base, bimodal peak, skewing to left or right, U shaped curve) were recorded.[(3,4)] A diagnosis was made about the category of anemia. The anemia was categorised based on RBC indices as: Normocytic normochromic, Microcytic hypochromic, Macrocytic normochromic when MCV was between 80-100fl, <80fl, >100fl respectively.[(5)] The RBC indices were seen in association with Histogram pattern. Normally positioned bell shaped RBC histogram was considered as normocytic normochromic anemia, left shift indicated Microcytic anemia, right shift indicated macrocytic anemia. A bimodal peak indicated dimorphic anemia and

Broad base with a left shift was considered as haemolytic anemia.

The correlation between the diagnoses made by Peripheral smear Vs RBC histogram, indices was analysed using Cramer's V (0.523) and Kappa statistics for agreement between the two methods.

Results

The RBC indices, histogram and peripheral smear of 500 patients having Hb less than 11.5 gm/dl were analyzed. The age group of patients included in this study ranged from 3 to 88 years. Majority of patients were between 41 and 50 years of age followed by 31- 40 yrs. and 21-30yr age group. Out of 500 patients 51.4% were females and 48.6% were males. Majority of females (72.3%) were in reproductive age group. After 40 years of age males were seen to be affected more than females.

In our study based on peripheral smear examination, the distribution of cases were as shown in Table 1

Table1: Shows distribution of anemia cases based on peripheral smear examination

Impression	No. of cases (n=500)	Percentage
Normocytic normochromic anemia	109	21.8%
Microcytic hypochromic anemia	230	46%
Macrocytic anemia	44	8.8%
Dimorphic anemia	88	17.6%
Hemolytic anemia	29	5.8%

RBC indices in study population: Hemoglobin in study population ranged from 2 to 10 gm % with mean of 7.7gm%. MCV in study population ranged from 49.5 fl to 139fl with mean of 81.09 fl. MCHC in study population ranged from 18.7 gm /dl to 42.6 g/dl with mean of 31.11 g/dl. MCH in study population ranged from 13.7 pg to 44.9 pg with mean of 25.2 pg. RDW in study population ranged from 12.4 % to 39.2 %with mean of 19.3%. PCV ranged from 9.5% to 65% with mean of 27.3 %.

Analysis based on RBC indices and Histogram: Of the 500 cases, majority (46.8%) were microcytic hypchromic anemia. Normocytic macrocytic, dimorphic and haemolytic were were 21%, 15.6%, 15.8% and 0.8% respectively. These are shown in Table 2

Table 2: Distribution of anemia cases based on RBC indices and Histogram

Diagnosis	No.of cases (n=500)	Percentage
Normocytic normochromic anemia	105	21%
Microcytic hypochromic anemia	234	46.8%
Macrocytic anemia	78	15.6
Dimorphic anemia	79	15.8%
Hemolytic anemia	4	0.8%

Normocytic normochromic anemia: RBC indices with other parameters are shown in Table 3. Reticulocyte percentage in study population ranged from 1 to3% with mean of 1.65%.

Table 3: Shows RBC indices in Normocytic normochromic anemia

RBC parameters	Min	Max	Mean
Hb	5.8	9.5	8.6
MCV	82	92	87
MCHC	30.2	33.9	32.3
MCH	19.5	32.9	28.8
RDW	12.4	20.9	15.9
PCV	17.8	65	33.5

Histogram analysis showed bell shaped curve in (95%) majority of cases (Fig. 1A). No shift to right or left was seen.

Microcytic Hypochromic Anemia: RBC indices with other parameters are shown in Table 4. Reticulocyte percentage in study population ranged from 1.3 to4% with mean of 2.2 %.

Table 4: Shows RBC indices in Microcytic hypochromic anemia

RBC parameters	Min	Max	Mean
Hb	2.0	10.0	7.6
MCV	49.5	76.0	71.6
MCHC	18.7	34.3	30.3
MCH	13.7	31.3	22.5
RDW	13.7	38.3	19.2
PCV	10.8	42.2	26.1

Most common histogram pattern seen in microcytic hypochromic was shift to left (87%) with broad base (Fig. 1B). Few cases showed bimodal pattern.

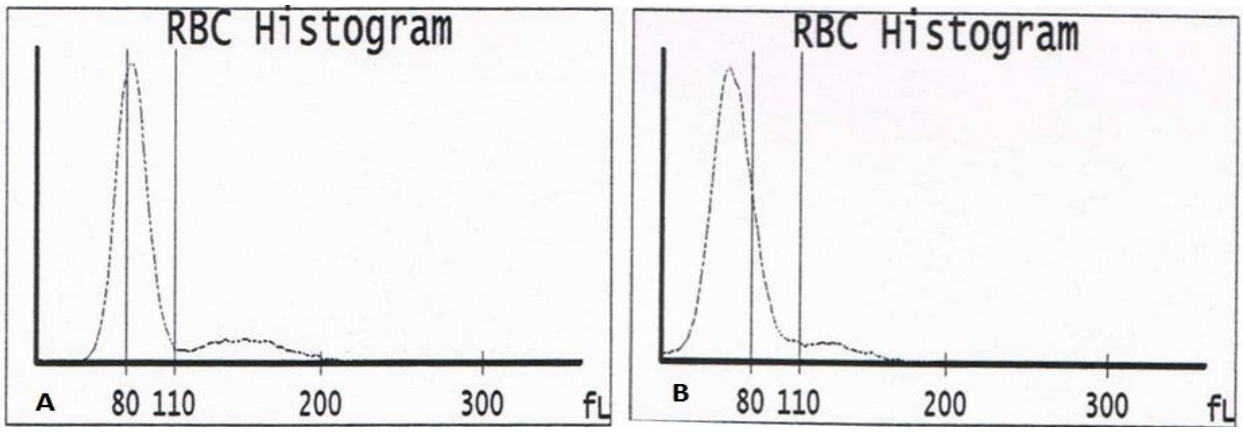

Fig. 1A: Shows normal histogram with bell shaped curve in normocytic normochromic anemia B Shows left shift of histogram with broad base in microcytic hypochromic anemia

Macrocytic anemia: RBC indices with other parameters are shown in Table 5. Reticulocyte percentage in study population ranged from 1.1 to3% with mean of 1.9 %.

Table 5: Shows RBC indices in Macrocytic normochromic anemia

RBC parameters	Min	Max	Mean
Hb	5.00	9.5	7.6
MCV	101	119	99.5
MCHC	30.7	34.5	32.1
MCH	19.5	33.1	29.98
RDW	15.4	27.3	18.3
PCV	15.5	39.9	26.5

Histogram pattern showed shift to right in majority (86.4) of cases (Fig. 2A). Also seen was bimodal pattern. Very few cases showed broad base curve.

Dimorphic anemia: RBC indices with other parameters are shown in table 6. Reticulocyte percentage in study population ranged from 1.2 to4% with mean of 2.1 %.

Table 6: Shows RBC indices in Dimorphic anemia

RBC parameters	Min	Max	Mean
Hb	4.5	9.4	7.8
MCV	60	110	83.8
MCHC	27.0	33.1	30.8
MCH	16.0	32.3	25.4
RDW	14.3	36.4	20.3
PCV	16.2	39.9	27.4

A bimodal peak with a broad base was seen in all the cases (Fig 2B). In addition to the bimodal pattern, 50 cases (63.6%) showed right shift with skewing of the histogram to the left indicative of combined nutritional deficiency (macrocytes with few microcytes). 27 cases showed a pure bimodal pattern indicative of anemia treated with hematinics/transfusion. One case showed left shift with skewing to right (microcytes predominantly with few macrocytes- Sideroblastic anemia and one case showed bimodal pattern with a combination of U shaped curve and bell curve indicating cold agglutinin disease.

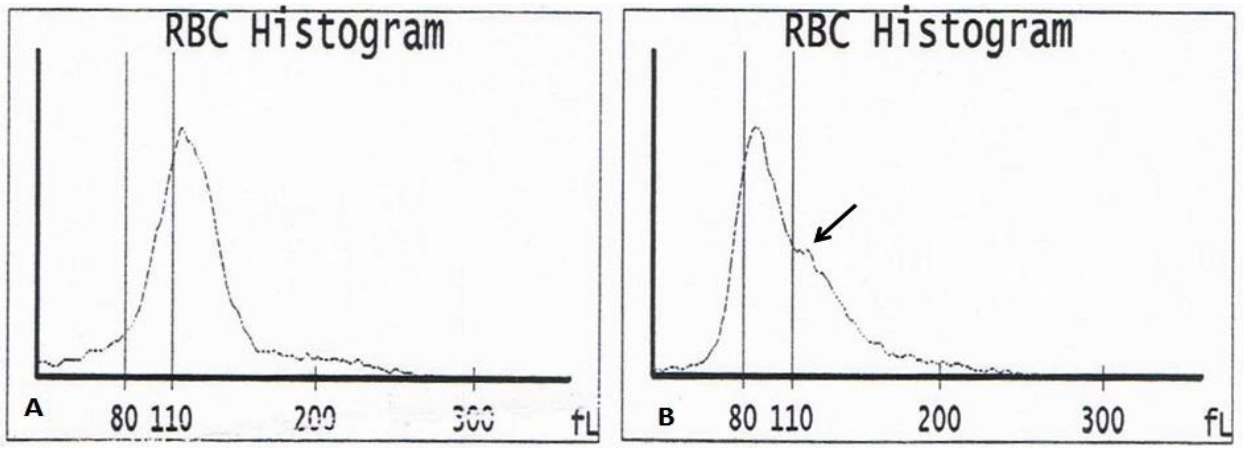

Fig.2A: Shows right shift of histogram in macrocytic anemia B Shows bimodal peak (arrow at second peak) indicating two cell popupaltions in dimorphic anemia

Hemolytic anemia: RBC indices and other parameters in hemolytic anemia are shown in table 7. Reticulocyte percentage in study population ranged from 1.2 to 5% with mean of 3.53%.

Table 7: Shows RBC indices in Hemolytic anemia

RBC parameters	Min	Max	Mean
Hb	2.8	9.4	6.4
MCV	50.3	139.5	71.9
MCHC	24.6	32.8	29.1
MCH	14.1	44.9	20.4
RDW	19.0	39.2	28.4
PCV	9.5	65	22.5

The histogram patterns seen in hemolytic anemia was shift to left with broad base (Fig3) with a few cases showed bimodal pattern and shift to right.

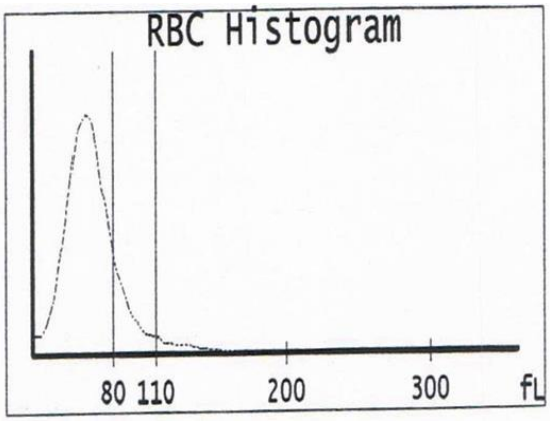

Fig. 3: Shows left shift of histogram with the curve beginning above the baseline in hemolytic anemia

Correlation of diagnosis of anemia based on peripheral smear vs Histogram and RBC indices analysis is shown in Table 8.

Table 8: Compares Peripheral smear analysis Versus Histogram, erythrocyte indices analysis

Type of Anemia	peripheral smear analysis	Histogram and RBC
Normocytic normochromic	109 (21.8%)	105(21%)
Microcytic hypochromic	230 (46%)	234(46.8%)
Macrocytic anemia	44 (8.8%)	78(15.6)
Dimorphic	88(17.6)	79(15.8%)
Hemolytic	29(5.8)	4(0.8%)

The impression made by Peripheral smear vs RBC histogram and indices were statistically analysed. The correlation between the diagnosis made by Peripheral smear vs RBC histogram and indices was analyzed using Cramer's V(0.523) which was statistically significant, $p<0.0001$ Kappa statistics for agreement between the two methods=0.518(moderate agreements), $p<0.0001$. As seen from table 8, Hemolytic anemia and macrocytic anemia were the two categories of anemia with a discrepancy in the impression analysed by Peripheral smear examination Vs histogram, RBC indices.

Discussion

A major public health problem worldwide, particularly among females of reproductive age in developing countries is anemia. Constantanio[(2)] and others have compared Red cell histogram and RBC indices while others have correlated RBC indices and peripheral smears.[(6)] But a correlation between all three parameters have been infrequently analysed. The present study was thus undertaken to correlate these in providing a better approach to the diagnosis of anemia.

In our study predominant age group was between 41 to 50 years and there was female preponderance. As in other studies by Japeth E and others.[(7)] On peripheral smear examination 80 out of 500 cases were normocytic normochromic anemia. On histogram analysis 76 cases showed position within normal limits and MCV ranged from 82-92fl. So diagnosis based on RBC indices and histograms were comparable to diagnosis with peripheral smear examination. It is comparable to study done by Kumar and others.[(6)]

Macrocytic anemia was seen in 44 out of 500 cases on peripheral smear examination. But histogram and RBC indices showed 78 cases as macrocytic anemia. This difference in the categorization was as a result of the interpretation of cases of hemolytic anemia as macrocytic anemia due to the presence of polychromatophils. Though MCV by automated blood cell counter is rarely inaccurate, hyperglycemia, marked leukocytosis and cold agglutinins may result in false elevation of the MCV. Few cases diagnosed as macrocytic on histogram and RBC indices analysis were diagnosed as dimorphic on peripheral smear examination.

The mild difference in the analysis of microcytic anemias by peripheral smear examination and by RBC indices/histogram can be explained by the presence of giant platelets and platelet clumps, fragmented RBCs in hemolytic diseases, when the autoanalyser considers it as microcyte. So peripheral smear rules out these errors. This study was in concordance with study done by Poonam and others.[(8)]

Only few cases were diagnosed as hemolytic anemia on Histogram and Indices analysis as compared to peripheral smear because fragmented RBCs were counted as microcytes and polychromatophils seen in hemolytic anemia were counted as macrocytes by cell counters. These findings points to the limitation of RBC histograms and RBC indices in the diagnosis of Hemolytic Anemias. Poonam and others have also reported such a limitation.[(8)] However the presence of a right skew in the RBC histogram and an elevated RDW with low PCV would alert to the presence of an element

of hemolysis in the anemia as seen in our study and by others.[9]

Histogram showed many broad based curves in dimorphic anemia in our study explained by the presence of multiple populations of cells of varying sizes (i.e. normocytic, microcytic and macrocytic). Different histogram patterns have been described to categories dimorphic anemia.[2] The Additional feature present along with a bimodal peak points to the diagnosis. A right shoulder corresponds to reticulocytosis, and a trail of erythrocyte population on the far right of the histogram correlates to red cell agglutination. A pure bimodal histogram is associated with therapeutic transfusion while a bimodal curve with a right shift and skewing towards left indicates a combined nutrional anemia. These findings seen in our study are in agreement with those described by[4] However since dimorphic anemia is associated with abnormal red cell populations, morphological findings should be correlated with the graphical and numerical data for better interpretation of results.

Thus, even in the age of molecular analysis, the blood smear examination remains an important diagnostic tool. Investigations done by using sophisticated modern equipment's for hematological disorders should be interpreted in the light of peripheral smear examination and clinical context. On the contrary, histogram gives information about subtle changes well in advance of numerical parameters and subjective evaluation, as in emerging iron deficiency in a case of megaloblastic anemia. Similarly a sequential histogram following administration of hematinics clearly shows the progressive appearance of a new erythrocyte population much before numerical parameters.

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