



CORRELATION OF PERIPHERAL SMEAR WITH RBC INDICES AND RBC HISTOGRAM IN THE DIAGNOSIS OF ANEMIA AT TERTIARY CARE CENTER

Hematology

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ABSTRACT

Introduction: 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. **Aims & Objective:** To correlate peripheral smear findings with RBC histogram patterns and RBC indices in the diagnosis of anemia. **Material And Methods:** 500 anemic patients with hemoglobin level $< 11.5 \text{ gm \%}$ on EDTA sample is collected and analysis done by ABX Micros 60 – HORIBA. An impression was made by the giemsa stain peripheral smear without referring to the histogram. The RBC histogram and RBC indices were analyzed and the anemia was categorized. The correlation between the diagnosis made by peripheral smear vs RBC histogram, indices was analyzed using Cramer's V. **Results:** Correlation of peripheral smear analysis with RBC indices and RBC histogram were done. On RBC histogram and RBC indices, 80(16%) cases showed normocytic anemia out of 76(15.2%) cases confirmed on peripheral smear. On RBC histogram and RBC indices 342(68.4%) cases showed microcytic anemia out of 329(65.8%) cases confirmed on peripheral smear. On RBC histogram and RBC indices, 25(5%) cases showed macrocytic anemia out of 11(2.2%) cases confirmed on peripheral smear. In dimorphic anemia, 62(12.4%) cases were confirmed on peripheral smear, but RBC histogram and RBC indices revealed 45(9%) cases. Hemolytic anemia, 22(4.4%) cases were confirmed on peripheral smear, but RBC histogram and RBC indices revealed 8 (1.6%) cases. The correlation between diagnosis made by peripheral smear vs RBC histogram and indices was analyzed using Cramer's V (0.667) which was statistically significant and the p-value is < 0.0001 . **Conclusion:** We concluded that diagnosis of anemia is supplemented by RBC indices and histogram while interpreting microscopic examination of peripheral smear, a good idea can be obtained from reviewing the histogram. However, visual microscopy is always superior for differentiating cells.

KEYWORDS

Anemia, RBC indices, RBC histogram, peripheral smear

INTRODUCTION:

Anemia is a condition in which red blood cells do not carry enough oxygen to the body's tissues. Preschool children, pregnant women, and adolescents constitute a high-risk group for anemia. Globally, anemia affects 1.62 billion people, corresponding to 24.8% of the population. The highest prevalence is in preschool-age children (47.4%), and the lowest prevalence is in men (12.7%). However, the population group with the greatest number of individuals affected is pregnant women (41.8%).^[1]

Over the past few years, complete blood count (CBC) by the automated hematology analyzers and microscopic examination of peripheral smears have complemented each other to provide a comprehensive report on patient's blood sample. The automated hematology analyzer has replaced the traditional manual methods for measuring various hematological parameters as the initial screening method in most of hospitals nowadays.^[2]

However, the peripheral smear examination has always been a cornerstone of diagnostic hematology. Analyzing the blood film has facilitated the interpretation of various hematological disorders and has been a major diagnostic tool for workup. The advent of automation has reduced subjective error and improved accuracy, precision, and safety in handling the blood sample. Nevertheless, microscopic examination is essential for primary calibration and presumptive diagnosis of anemia, leukemia, and other related disorders.^[3]

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.^[4]

RBC Histograms are graphical representations of cell frequencies versus sizes. In a homogenous cell population, the curve assumes a symmetrical bell-shaped or Gaussian distribution. The peak of the curve should be within the normal MCV of 80.0-100.0 fl.^[5] The red cell distribution curve will get wider as the red cells vary more in size, a narrow distribution curve indicates a homogenous population of red

cells; the wider the distribution curve indicates a more heterogeneous population of red cells.^[6]

Aims And Objectives:

1. To diagnose the type of anemia by peripheral smear examination.
2. To diagnose the type of anemia by RBCs indices and RBCs histogram.
3. To correlate peripheral smear findings with RBC histogram patterns and RBC indices in the diagnosis of anemia.

MATERIAL AND METHODS:

It was a cross sectional-observational study conducted at the hematology lab of a department of pathology, a tertiary care center affiliated with the medical college from March to April 2020. The study was conducted on 500 samples collected in an EDTA vacutainer for complete blood count at an indoor hematology laboratory. Age and sex were recorded from every patient.

Inclusion Criteria:

- HB level $< 11.5 \text{ gm \%}$ in all age and sex groups.

Exclusion Criteria:

- HB level $> 11.5 \text{ gm \%}$ in all age and sex groups.
- HB level $< 11.5 \text{ gm \%}$ with hematological malignancy

The blood samples of all the study cases were drawn from the antecubital vein using a 5ml syringe and immediately collected and mixed in EDTA vacutainers. Sample were run within 2 hours of sample collection. Analysis of the blood samples was done by a 3-part Automated hematology analyzer **ABX Micros 60 – HORIBA**. Sample with hemoglobin $< 11.5 \text{ gm \%}$ were selected and the following data were collected: Hemoglobin, PCV, RBC count, MCV, MCH, MCHC, RDW, Total WBC count & Platelet count. Peripheral smears were made from the EDTA sample, fixed, and stained by standard protocol with Giemsa for red cell morphology examination. The retic count of all cases was also recorded.

Correlation of peripheral smear with RBC indices & histogram:

- Peripheral smear of all cases was evaluated for RBC morphology making an impression of type of anemia without referring to RBC histogram and indices.
- RBC histogram and RBC indices were analyzed, and anemia was categorized based on RBC indices as normocytic normochromic, microcytic hypochromic, and macrocytic when MCV between 80-100 fl, <80 fl and >100 fl respectively.
- RBC indices were seen in association with the histogram pattern.
- Histogram was analyzed for their position: Normal bell-shaped curve: normocytic normochromic, Left shift: microcytic anemia, Right shift: macrocytic anemia, Bimodal peak: Dimorphic anemia, Broad base with left shift and Curve beginning from the baseline: hemolytic anemia.

Statistical Analysis: Collected data was entered in a Microsoft Excel sheet and data was analyzed using a software statistical package for social science version-23.0(SPSS). The correlation between diagnosis made by peripheral smear versus RBC histogram and indices was analyzed by using Cramer's V.

RESULTS:

The RBC indices, histogram, and peripheral smear of 500 patients having HB < 11.5 gm/dl were analyzed. In our study, the majority age group was 21-30 years with 39.6% followed by the 31-40 year age group with 17.2%. Female were 69.4% and males were 30.6%. Out of 347 females, the majority 51.6% of females were in the reproductive age group (21-50 years). According to hemoglobin concentration, mild (>10 gm%), Moderate(7-10 gm%) and severe (<7 gm%).^[7] The majority of cases 271(54.2%) have moderate anemia followed by mild anemia in 120(24%) of cases. 109(21.8%) cases had severe anemia with hemoglobin <7 gm%.

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

Table-1: Distribution Of Anemia Cases Based On Peripheral Smear Examination

Diagnosis	No. of cases	Percentage (%)
Normocytic Normochromic anemia	76	15.2
Microcytic hypochromic anemia	329	65.8
Macrocytic anemia	11	2.2
Dimorphic anemia	62	12.4
Hemolytic anemia	22	4.4
Total	500	100

RBC Indices In The Study Population: In the present study, hemoglobin ranged from 2 to 11.4 gm% with a mean of 8.34 gm%. PCV ranged from 5.6 % to 37.6 % with a mean of 25.3 %. MCV ranged from 42 to 132 fl with a mean of 75.69 fl. MCH in the study population ranged from 12.2 pg to 47.7 pg with a mean of 25.1 pg. MCHC ranged from 25 gm/dl to 38.1 gm/dl with a mean of 32.9 gm/dl. RDW ranged from 11.3% to 33.1% with a mean of 17.44 %.

Analysis Based on RBC histogram and RBC indices: Table 2 shows out of 500 cases, maximum cases showed microcytic hypochromic anemia 342(68.4%), 80(16%) cases showed normocytic normochromic, 45(9%) cases showed dimorphic anemia, 25(5%) cases showed macrocytic anemia and 8(1.6%) cases showed hemolytic anemia.

Table-2 distribution of anemia cases based on RBC histogram and RBC indices

Diagnosis	No. of cases	Percentage (%)
Normocytic Normochromic anemia	80	16
Microcytic hypochromic anemia	342	68.4
Macrocytic anemia	25	5
Dimorphic anemia	45	9
Hemolytic anemia	8	1.6
Total	500	100

Normocytic Normochromic Anemia: RBC indices and other parameters are shown in Table 3. Among the total 76(15.2%) cases of normocytic anemia on peripheral smear, the majority showed normal histogram curve 69(90.8%). Few cases 4(5.3%) have a broad base and 3(3.9 %) cases show a left shift of histogram. Reticulocyte count in the study population ranged from 0.2 to 1.4% with a mean of 0.8%.

Table– 3: RBC indices in normocytic normochromic anemia

RBC parameters	Min	Max	Mean
Hb	7.9	11.4	10.5
PCV	22.9	35.5	31.0
MCV	78	96	85.7
MCH	25.7	33.9	29.1
MCHC	31.2	37.7	34.2
RDW	11.3	18	14.2

Microcytic Hypochromic Anemia: RBC indices and other parameters are shown in Table 4. Among the total 329(65.8%) cases of microcytic anemia on peripheral smear, most of the cases 258(78.42%) showed left shift, followed by broad-based curve 53(16.11%), Bimodal 9(2.74%), normal curve 6(1.82%) and 3(0.91%) short peak. Reticulocyte count in the study population ranged from 0.2 to 2.4% with a mean of 1.2%.

Table- 4 : RBC indices in microcytic hypochromic anemia

RBC Parameters	Min	Max	Mean
Hb	2.2	11.4	8.29
PCV	6.3	37.6	25.5
MCV	42	96	68.8
MCH	12.2	31.6	22.3
MCHC	25	37.5	32.4
RDW	11.5	31.7	17.4

Macrocytic Anemia: Table 5 shows RBC indices with other parameters. Among the total 11(2.6%) cases of macrocytic anemia on peripheral smear, most of the cases 8(72.7%) showed right shift and 3(27.3%) cases showed a broad base curve. Reticulocyte count in the study population ranged from 0.4 to 1.6% with a mean of 0.9%.

Table- 5: RBC indices in macrocytic anemia

RBC parameters	Min	Max	Mean
Hb	2.8	11	8.1
PCV	8.7	29.8	23.5
MCV	98	123	110.2
MCH	32.2	45.2	38.1
MCHC	30.8	36.7	34.4
RDW	13.5	19.8	16.9

Dimorphic Anemia: Table 6 shows RBC indices with other parameters. Among the 62(12.4%) cases of dimorphic anemia in the peripheral smear, 23(37.1%) cases had a bimodal curve, followed by 22(35.4%) cases that had a broad base curve. Right shift 12(19.4%) cases, 4(6.5%) cases had left shift and 1(1.6%) cases had normal curve. Reticulocyte count in the study population ranged from 0.2 to 2.8% with a mean of 1.6%.

Table-6: RBC indices in dimorphic anemia

RBC parameters	Min	Max	Mean
Hb	2.0	10.9	6.2
PCV	5.6	31.9	18.3
MCV	62	132	93.1
MCH	19.3	47.7	31.9
MCHC	28.4	38.1	34.3
RDW	14.6	33.1	21.3

Hemolytic Anemia: The table shows RBC indices with other parameters. Among the 22(4.4%) cases of hemolytic anemia on peripheral smear, most of the cases showed broad base 10(45.5%), followed by 9(40.9%) cases had left shift, 2(9.1%) cases had a right shift and 1(4.5%) cases had a bimodal curve. Reticulocyte count in the study population ranged from 0.7 to 6.9% with a mean of 2.24%.

Table – 7: RBC indices in hemolytic anemia

RBC parameters	Min	Max	Mean
Hb	2.3	11.4	7.3
PCV	7	31.7	22.6
MCV	53	119	78.3
MCH	17	30.2	25.5
MCHC	29.5	36.9	32.5
RDW	12.5	27.7	18.03

The correlation of RBC histogram and RBC indices with peripheral smear in the diagnosis of anemia is shown in Table 8.

Table-8: comparison peripheral smear analysis versus histogram,

RBC indices analysis

Type of anemia	Peripheral smear analysis	Histogram and RBC indices
Normocytic Normochromic anemia	76(15.2%)	80(16%)
Microcytic hypochromic anemia	329 (65.8%)	342(68.4%)
Macrocytic anemia	11 (2.2%)	25 (5%)
Dimorphic anemia	62 (12.4%)	45 (9%)
Hemolytic anemia	22 (4.4%)	8(1.6%)

Statistical Analysis:

The impression made by peripheral smear vs RBC indices and histogram were statistically analyzed with a statistical package for the social science version-23.0 software. The correlation between diagnosis made by peripheral smear vs RBC histogram and indices was analyzed using Cramer's V(0.667) which was statistically significant and $p < 0.0001$.

DISCUSSION:

The present study intends to compare RBC histogram pattern and RBC indices with morphological features seen on peripheral smear examination; so that laboratory personnel and clinicians can use this with sufficient accuracy to presumptively diagnose morphological classes of anemia directly from the automated cell counter forms and correlate with morphological features of peripheral smear examination.

Normocytic Normochromic Anemia: On peripheral smear examination, 76(15.2%) cases out of 500 cases were normocytic normochromic anemia and RBC indices & histogram analysis showed 80(16%) cases. On histogram analysis 69(90.8%) cases showed a curve within the normal position, and MCV ranged from 78-96 fl. So, diagnosis based on RBC indices and histograms was comparable to diagnosis with peripheral smear examination. It is comparable with Karishma A et al^[8].

Microcytic Hypochromic Anemia: On peripheral smear examination 329(65.8%) cases were seen and on histogram and RBC indices revealed 342(68.4%) cases. Histogram analysis showed left shift in 258(78.42%) cases. MCV ranged from 42-96 fl. The mild difference in the analysis of microcytic anemias by peripheral smear examination and by RBC indices/histogram can be elucidated by the presence of giant platelets and platelet clumps, fragmented RBCs in hemolytic diseases, when the autoanalyzer considers it as microcyte. So peripheral smear rules out these errors. Our findings were in concordance with the study done by Sandhya V et al^[9] and Aseem Jain et al^[1].

Macrocytic Anemia: On peripheral smear examination 11(2.2%) cases were seen and on histogram and RBC indices revealed 25(5%) cases. On histogram analysis showed a Right shift in 8(72.7%) cases. MCV ranged from 98-123 fl. There is a notable difference in findings of peripheral smear and RBC indices. It may be due to the interpretation of hemolytic anemia as macrocytic anemia as a result of polychromatophils giving the high value of MCV. However, MCV by automated cell analyzer is rarely inaccurate. Hyperglycemia, cold agglutinins, and leukocytosis may result in false elevation of MCV. Few cases diagnosed as macrocytic on histogram and RBC indices analysis were diagnosed as dimorphic on peripheral smear examination. Our findings were in concordance with the study done by Poonam et al^[10] and Sarita Choudhary et al^[11].

Dimorphic Anemia: On peripheral smear examination 62(12.4%) cases were seen and on histogram and RBC indices revealed 45(9%) cases. On histogram analysis bimodal curve 23(37.1%) and broad base curve 22(35.4%). MCV ranged for dimorphic was varies from 62-132 fl. The bimodal curve is associated with therapeutic transfusion, and hematinic agent response to microcytic and macrocytic anemia. It is also seen in early iron deficiency developing microcytic population and folate/vitamin B12 deficiency developing macrocytic population. A bimodal curve with right skewing indicates reticulocytosis and a tail of erythrocytes population on the far right of the histogram correlates with red cell agglutination. A bimodal curve with a right shift & skewing towards the left indicates combined nutritional deficiency. The broad base curve can be explicated by the presence of multiple populations of cells of varying sizes (i.e. normocytic, microcytic, and

macrocytic). The study was in concordance with Sandhya V et al^[9] and Aseem Jain et al^[1]. However, since dimorphic anemia is related to abnormal red cell populations, morphological findings should be correlated with the graphical and numerical data for better interpretation of results.^[9]

Hemolytic Anemia: On peripheral smear examination 22(4.4%) cases were seen and on histogram and RBC indices revealed 8(1.6%) cases. Histogram curve of these 8(1.6%) cases starting above the baseline. On histogram analysis, the majority showed broad base 10(45.5%) and left shift 9(40.9%). MCV ranged from 53 to 119 fl. Few cases were diagnosed on histogram because fragmented RBCs were counted as microcytes and polychromatophils were counted as macrocytes by cell counter. The similar limitations of RBC histogram and RBC indices in the diagnosis of anemia were also reported by Sandhya V et al^[9] and Garg et al^[3]. 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^[4].

Thus, we can see histograms with RBC indices are useful diagnostic aids in microcytic hypochromic anemia and normocytic normochromic anemia. However, macrocytic, dimorphic, and hemolytic anemia showed different histogram patterns from simple to complex curves. Therefore, in all patients with decreased hemoglobin peripheral smear examination is a must for identifying red cell changes. Bain^[12] in her review on the place of peripheral smear examination in the era of automation in 2005 stated that even in the age of molecular analysis, blood smear remains an important diagnostic tool and sophisticated modern investigations of hematological disorders should be interpreted in the light of peripheral blood smear as well as clinical context.

CONCLUSION:

Our study showed a significant correlation of peripheral smear with RBC indices and histogram in the diagnosis of microcytic hypochromic anemia and normocytic normochromic anemia. However, macrocytic anemia, dimorphic anemia, and hemolytic anemia showed different histogram patterns. We concluded that diagnosis of anemia is supplemented by RBC indices and histogram while interpreting microscopic examination of peripheral smear, a good idea can be obtained from reviewing the histogram. However, visual microscopy is always superior for differentiating cells. The benefits of manual scans are the ability to identify clinically significant morphology of cells that are not quantifiable by instruments. Thus, even in the era of automation, peripheral smear examination remains the definitive diagnostic test for evaluation.

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