ORIGINAL ARTICLE ASIAN JOURNAL OF MEDICAL SCIENCES

Correlation of automated cell counter RBC histograms and peripheral smear in anemia typing

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Submission: 29-10-2022 Revision: 29-12-2022 Publication: 01-02-2023

ABSTRACT

Background: Red blood cell (RBC) indices and histogram obtained from automated hematology gives an idea about the morphological changes in anemia and therefore forms the fundamental process for diagnosis of anemia. Along with the peripheral smear, they can be used for anemia typing and to interpret the cause of anemia. **Aims and Objectives:** (1) Interpretation of histograms in normal persons and patients with different types of anemia. (2) Anemia typing based on RBC histograms complemented with Peripheral smear examination. **Materials and Methods:** The present prospective study is conducted in Haematology section of Department of Pathology, Shyam Shah Medical College and Sanjay Gandhi Medical Hospital Rewa, Madhya Pradesh. A total of 1000 patients blood sample is collected for complete blood count and anemia typing irrespective of age and gender and examined over a period of 1 year from August 2021 to July 2022. The patient's blood sample is collected in EDTA vacutainer and analyzed on the basis of histogram obtained and peripheral smear slide prepared. **Results:** In the present study, we noted that the predominant age group affected was 20–30 years. Peripheral smear finding shows normocytic normochromic anemia was seen in 6% cases (60/1000), microcytic hypochromic anemia seen in 24.2% cases (242/1000), macrocytic anemia seen in 14% cases (140/1000), dimorphic anemia seen in 50.2% cases (502/1000), and hemolytic anemia seen in 5.6% cases (56/1000). This peripheral smear finding shows well correlation with findings of histogram pattern and RBC indices, where normocytic normochromic seen in 6.4% of cases (64/1000), microcytic hypochromic anemia seen in 24.4% of cases (244/1000), macrocytic anemia seen in 13.8% of cases (138/1000), dimorphic anemia seen in 49.5% of cases (495/1000), and hemolytic anemia seen in 5.9% of cases (59/1000). The most common anemia is dimorphic anemia (combined nutritional deficiency) in 49.5% cases followed by microcytic hypochromic anemia in 24.4% of cases based on histogram findings along with RBC indices. **Conclusion:** Histogram guides a technologist about the cases that need actual detailed peripheral smear examination by experts. Our study shows a well correlation in findings of automated hematology analyzer with the microscopic examination. Histogram alone could be used as screening method and when combined with peripheral blood smear findings, they act as useful supplement and by correlating findings of both methods, we could diagnose majority of anemia.

Key words: Histogram; Anemia; Peripheral smear

Access this article online

Website: http://nepjol.info/index.php/AJMS

DOI: 10.3126/ajms.v14i2.49128

E-ISSN: 2091-0576

P-ISSN: 2467-9100

INTRODUCTION

The foundation of every laboratory examination is the complete blood count (CBC), which includes the differential lymphocyte count (DLC). The CBC and DLC are crucial for diagnosing anemia, acute and chronic illnesses, white cell disorders, leukemia, and platelet disorders.[1] In most laboratory setup today, histogram analysis is often a

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neglected and underrated part of the automated hemogram which if interpreted well, has significant potential to provide diagnostically relevant information even before higher level investigations are ordered and may be a time saving tool.[2]

During past 25 years blood cell analysis has progressed from manual procedures to highly automated instruments.[3] The red blood cell (RBC) histogram visualizes particle size distribution that plays a critical role in the initial screening and detection method for hematological disorders in current clinical settings.[4,5] Here, we describe and discuss in detail the utility of RBC histograms in various hematological disorders in routine clinical practice.

The advent of automation has reduced subjective errors, improved accuracy, precision, and safety in handling of blood samples. Nevertheless, microscopic examination is essential for primary calibration and for presumptive diagnosis of anemia, leukemia, and other related disorders.[1]

Aims and objectives

The present study was performed with aim of Interpretation of histograms in normal persons and patients with different types of anemia and anemia typing based on RBC histograms and RBC indices complemented with peripheral smear examination.

MATERIALS AND METHODS

The present study is conducted in Hematology section of Department of Pathology, S.S.M.C and S.G.M.H Rewa, Madhya Pradesh. After taking ethical clearance from the Institutional Ethical Committee, a total of 1000 patients blood sample were collected for CBC and anemia typing and examined over a period of 1 year from August 2021 to July 2022. The patients are selected based on the following criteria.

Inclusion criteria

1. All cases of anemia are included in the present study which were decided on the basis of the WHO 2011 Criteria to diagnose Anemia:
   - Children ½–5 years (Hb <11)
   - Children 5–11 years (Hb <11.5)
   - Children 11–15 years (Hb <12)
   - Pregnant females (Hb <13)
   - Non-pregnant females (15 years and above) (Hb <13).
   - Men (15 years and above) (Hb<13).

Exclusion criteria

The following criteria were excluded fron the study:

1. Inadequate quantity of blood sample for automatic hematology analyzer
2. Patients suffering from malignancies are excluded.

The microscopy findings obtained on peripheral smear examination were compared with histogram chart on the Mindray hematology cell counter. The position of RBC histogram (normal, left shift, and right shift) and the shape of RBC histogram (normal bell shape or Gaussian, widened base, bimodal peak, skewing to left or right, and U-shaped curve) recorded.

Categorization of anemia is done on the basis of RBC indices of hematology analyzer was done as:

1. Microcytic hypochromic anemia shows the left shift in RBC histogram
2. Normocytic normochromic anemia is seen as normal positioned bell shaped RBC histogram
3. Macrocytic anemia is seen as the right shift in RBC histogram
4. Dimorphic anemia appears as bimodal peak
5. Hemolytic anemia is seen as broad base with left shift.

RESULTS

The present study titled "Correlation of Automated Cell Counter RBC Histograms and Peripheral Smear in Anemia Typing" was conducted on 1000 patients blood sample received for CBC and anemia typing in the Hemato-Pathology Department of a tertiary care institution in Central India. Gender-wise distribution of cases is shown in Table 1. Out of total 1000 cases, female predominance was seen in 61% (610/1000) of all cases and male population consisted of 39% (390/1000) with female-to-male ratio of 1.6:1.

In the present study, most of the samples belonging to the age between 20 and 30 years (45%), followed by 30–40 years (23%). The least common age group in the current study was 40–50 years (7%) (Table 2). The reason maybe probably attributed to significant number of reproductive age females belonging to this age group and also adolescent and adult age is period of intense growth and development thereby requiring high iron demands.

In the current study, out of 1000 cases that were observed in peripheral smear, most common finding is dimorphic anemia (Figure 1) seen in 50% (502/1000) of total cases followed by microcytic hypochromic anemia (Figure 2)

Table 1: Gender-wise distribution of anemia cases

Gender	Frequency (n=1000)	Percentage
M (Male)	390	39
F (Female)	610	61
Total	1000	100

Table 2: Age-wise distribution of anemia cases

Distribution	Newborn-20	20–30	30–40	40–50	>50	Total
Frequency	155	455	230	65	95	1000
Percentage	16	45	23	7	9	100

Table 3: Distribution of cases per type of anemia based on peripheral smear examination

Types of anemia	Percentage
Normocytic normochromic	60 cases (6)
Microcytic hypochromic	242 cases (24.2)
Macrocytic	140 cases (14)
Dimorphic	502 cases (50.2)
Hemolytic	56 cases (5.6)
Total	1000 cases (100)

Table 4: Comparative study based on histogram findings

Types of histogram	Frequency (n=1000)	Percentage
Normal curve	70	7
Left shift	272	27
Right shift	136	14
Broad base	376	38
Bimodal peak	146	14

Table 5: Categorization of anemia based on RBC indices and histogram curve

Types of anemia	Percentage
Normocytic normochromic	64 cases (6.4)
Microcytic hypochromic	244 cases (24.4)
Macrocytic	138 cases (13.8)
Dimorphic	495 cases (49.5)
Hemolytic	59 cases (5.9)
Total	1000 cases (100)

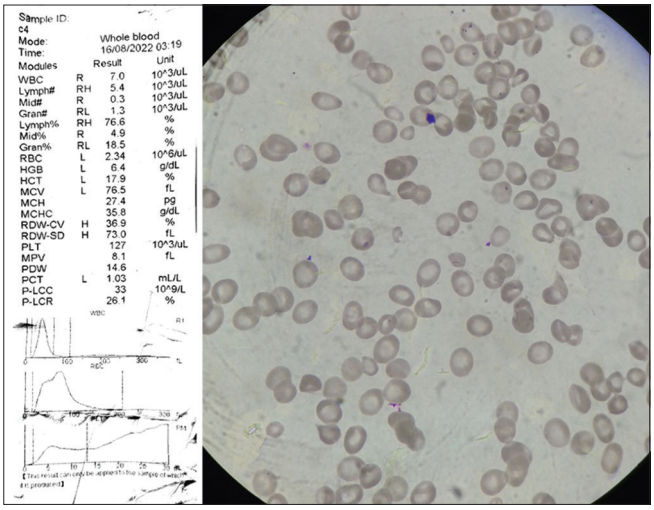

Figure 1: Histogram showing bimodal peak and peripheral smear findings of dimorphic anemia

in 24.2% (242/1000) followed by macrocytic anemia (Figure 3) in 14% (140/1000). Normocytic normochromic

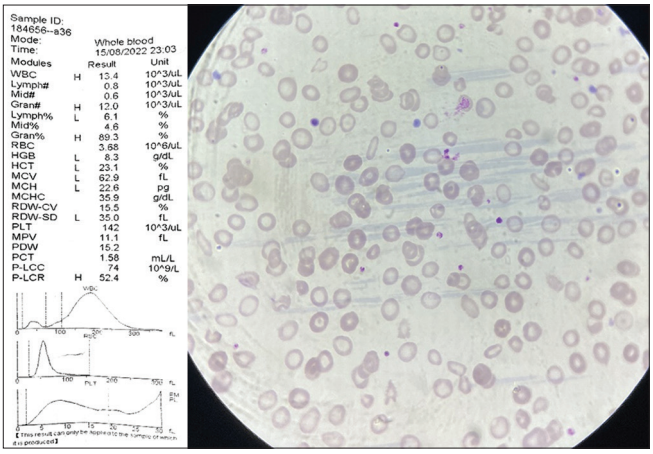

Figure 2: Histogram showing the left shift and peripheral smear of microcytic hypochromic anemia

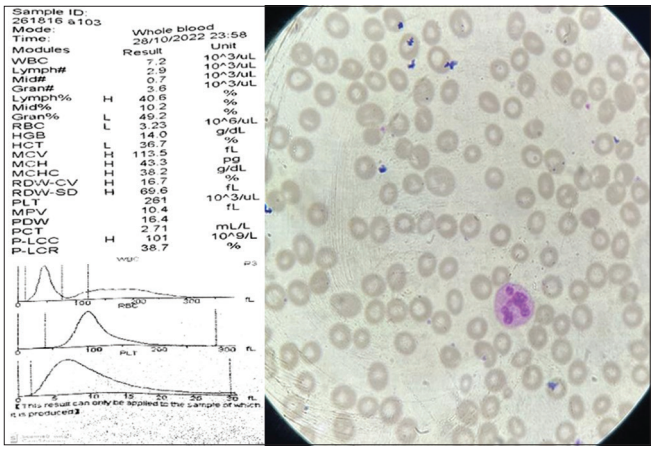

Figure 3: Histogram showing the right shift and peripheral smear of macrocytic anemia

anemia was seen in 6% (60/1000) and hemolytic anemia was least common finding observed in only 5.6% (56/1000) of total cases (Table 3).

Table 4 shows distribution of RBC histogram curve. Most commonly observed is broad base pattern (38%) followed by the left shift (27%). Bimodal peak and right shift are observed in 14% of cases each. Least common histogram pattern observed is a normal curve seen in only 7% of total cases.

The anemia typing is further done on the basis of histograms pattern obtained and RBC findings. Out of 1000 cases, maximum number of cases shows dimorphic

Table 6: Correlation of peripheral blood findings with histogram patterns

Investigation	Normocytic normochromic anemia count (percent)	Microcytic hypochromic anemia count (percent)	Macrocytic anemia count (percent)	Dimorphic anemia count (percent)	Hemolytic anemia count (percent)	Total
Peripheral smear finding	60 (6)	242 (24.2)	140 (14)	502 (50.2)	56 (5.6)	1000
Histogram pattern and RBC indices	64 (6.4)	244 (24.4)	138 (13.8)	495 (49.5)	59 (5.9)	1000

RBC: Red blood cells

Table 7: Comparison of age group for anemia in previous studies

Age groups (years)	Present study (%)	Korgaonker and Shashidhar[9] 2019 (%)
<30	61	46.3
31–40	23	14.9
41–50	7	14.1
51–60	9	9.98
>60	-	14.37

Table 8: Comparison of histogram pattern in previous studies

Histogram pattern	Present study (%)	Sandhya and Muhasin 2014 (%)	Chavda et al.,[2] 2015 (%)
Normal curve	7	15	19
Left shift	27	30	27
Right shift	14	6	7
Broad base	38	40	38
Bimodal peak	14	4	3
Short peak	-	5	6

anemia seen in 49.5% of cases followed by microcytic hypochromic anemia in 24.4% cases. Macrocytic anemia seen in 13.8% of cases, normocytic normochromic and hemolytic anemia were observed in 6.4% and 5.9%, respectively (Table 5).

Table 6 shows importance of histogram and red cell indices in diagnosis of anemia and how close they are to the actual diagnosis made after peripheral smear examination.

All types of anemia correlated well with the histogram pattern except for dimorphic anemia, which showed different type histogram curve. Dimorphic blood picture is dual population of microcytic and normocytic or normocytic and macrocytic red cell or mixture of small, normal or large cell of different size and forms with or without normal RBC indices which can mislead the diagnosis if we rely on automated values alone. Nutritional anemia, recent blood transfusion or therapeutic response to nutritional anemia, and sideroblastic anemia are all possible causes of dimorphic blood images. To determine the specific cause, a complete examination is required. Hence, it is very important to examine peripheral blood smear (PBS), RBC indices, and histogram in all patients of anemia.

DISCUSSION

The effect of a vast collection of facts represented as a visual representation is significantly greater than the impact of numbers alone. These data can take numerous forms in hematology, one of which is the RBC histogram. This study is done to define the utility of histogram with RBC indices in anemia typing and to establish it as a routine diagnostic tool in laboratories (Tables 7 and 8).

Sex distribution

The total number of cases of anemia in our study was 1000. Female predominance was observed in the present study with 61% of the cases which was comparable to the past similar research of Singhal et al., (64.9%)[6] and Garg et al., (62.9%).[7]

Age

In the present study, the most affected age group was 20–30 years followed by 30–40 years which was comparable to the results of similar studies done by Korgaonker and Shashidhar[8] Kumar et al.,[9] Cook et al.[10]

Our study of RBC histogram showed normal curve (7%), left shift (27%), right shift (14%), broad base (38%), and bimodal (14%) and these findings regarding to RBC histogram were also correlated with other studies such as Sandhya and Muhasin,[11] Chavda et al.,[2] Rao et al.,[12] and Shrivastav et al.[13]

The most common type of anemia observed in our study was dimorphic anemia (combined nutritional deficiency) followed by microcytic hypochromic anemia indicative that the most common anemia observed in Vindhya region population is combined nutritional deficiency which is in contrast with studies by Sandhya and Muhasin,[11] Chavda et al.;[2] and Rao et al.,[12] which shows microcytic hypochromic anemia as the most common distribution pattern.

Limitations of the study

Practically, since dimorphic anemia is usually associated with abnormal red cell populations, morphological findings

should be correlated with the graphical and numerical data for better interpretation of results. Recent blood transfusion may be a limitation for typing of anemia.

CONCLUSION

RBC histogram is an important and extremely time saving tool of diagnosis when correct interpretation of curve is combined with findings of blood count parameters such as red cell distribution width and red cell indices.[13] Histograms guides a technologist about the cases that need actual detailed peripheral smear examination by experts.[14] Our study shows a well correlation in findings of automated hematology analyzer with the microscopic examination. Histogram alone could be used as screening method and when combined with PBS findings, they act as useful supplement and by correlating findings of both methods, we could diagnose majority of anemia.

ACKNOWLEDGMENT

The authors would like to thank the department of pathology, with all the technical and non-technical staff for their co-operation and co-ordination throughout the period of the study.

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Authors' Contributions:
HM- Concept and design of the study, prepared first draft of manuscript; **PCS-** Concept, coordination, statistical analysis and interpretation, preparation of manuscript and revision of the manuscript; **RG-** Interpreted the results; reviewed the literature and manuscript preparation; **AG-** Preparation of manuscript, interpretation, and revision of the manuscript; **SKS-** Revision of the manuscript.

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Source of Support: Nil, **Conflict of Interest:** None declared.