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THE IMPORTANCE OF HISTOGRAMS IN DIAGNOSIS OF ANAEMIA

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ABSTRACT

Anaemia is a widespread public health issue, with extremely high prevalence rates in India. The RBC histogram, in conjunction with other CBC parameters, is a valuable tool for diagnosing and treating variety of RBC disorders. A histogram study can become a new parameter in diagnosing anaemia. The goal of this research is to produce a guide that will allow laboratory staff and physicians to accurately diagnose morphological classes of anaemia based on automated haematology cell counter forms.This is a cross-sectional investigation. A total of 500 blood samples from patients with haemoglobin values of <11gm%, irrespective of their age and sex were collected in K2 EDTA bulb and the samples were run in Siemens Advia 120-2120i automated haematology analyzer in the Central Laboratory of a tertiary care Hospital in Aurangabad from December 2016 to October 2018. A normal curve was present in 130 out of 153 cases of

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normocytic anaemia, a left shift curve was present in 148 out of 210 cases of microcytic anaemia, a right shift curve was present in 19 out of 25 cases of macrocytic anaemia, a left shift curve was present in 44 out of 100 cases of dimorphic anaemia, and five left shift and five broad base curves were present in 10 cases of hemolytic anaemia. With advancing technology, it has now become easier to evaluate the type of anaemia with the help of cell counters, especially with one look at histograms and scattergrams.

Keywords: RBC, CBC, cell counter, scattergram

INTRODUCTION

Anaemia affects an estimated population of 2.36 billion globally, particularly women and children [1] .One of the nations with high prevalence rates is India, where diagnosing anaemia is a constant challenge [2].

The presenting symptoms and the diagnosis of anemia are usually non-specific. The initial morphological classification and its correlation with red blood cell indices and morphological attributes are important in determining the next steps for clinical management [3]. In recent years, microscopic analysis of peripheral smears and automated analyzers for complete blood counts (CBCs) have complemented each other to offer a thorough report on a patient's blood sample. Since hematology has become more automated, it is now possible to subcategorize the different anaemias quickly and affordably by using the red cell parameters provided by the analyzers. A histogram can give laboratories valuable information for tracking the dependability of the analyzer's results, researching the possible cause or causes of the inaccurate automated results, and making an educated guess to the diagnosis. This was previously difficult to obtain without a blood smear examination, but a red cell histogram may now be used to help detect it [4].When presented as a visual image instead of only numbers, a large collection of data can communicate

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information more effectively. It follows that one histogram graph is equivalent to 1000 words. A histogram analysis can be used as a new parameter for diagnosing anaemias, and technologists may find it helpful in prioritizing cases and expediting the laboratory's sample disposal process.

METHODOLOGY

Over the course of three years, 500 CBC samples were chosen. These samples came from the daily workload that was chosen at random. Informed consent was taking using the consent form. The following was carried out in accordance with the laboratory policy: Demographic information such as the patient's age, sex, and clinical status were gathered from the lab request. Two milliliters of peripheral venous blood were drawn into potassium ethylene diamine tetra acetic acid (K2 EDTA) vacutainers under strict aseptic conditions and delivered to the lab.

Using the Advia 120-2120i system, automated CBC and differential counts were carried out on every sample within an hour of collection. During the investigation, capped samples were kept at room temperature. Red cell distribution width was reported by the system as the coefficient of variation from the mean distribution width corpuscular volume (RDW-CV) as well as standard deviations from the mean red cell distribution width (RDW-SD). To ensure high performance, quality assurance as well as quality control procedures were followed. Regular calibrations of the hematology analyzer were performed using standardized calibrators. Every day whole-blood controls were employed to track the analyzer's effectiveness. Patient samples were never used until Westgard rules- based quality control was achieved.

Ethical clearance has been obtained from the Mahatma Gandhi Mission's Ethics Committee for Research on Human Subjects, MGM Medical College and Hospital, Aurangabad, Maharashtra with the IEC number MGM ECRHS/2016/05.

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Statistical Software for Social Sciences (SPSS) (IBM) Version 21 was used to enter the obtained data and produce descriptive statistical measures like mean, standard deviation, percentages, and so forth.

RESULTS

The Central Clinical Laboratory, Department of Pathology, tertiary care Hospital, Aurangabad is where the current investigation was conducted. 500 patients with hemoglobin levels less than 11 gm/dl had their RBC indices, peripheral smear, and histogram examined and correlated. The following outcomes were attained:

Following their selection for the study, all patients who met the inclusion and exclusion criteria had their red blood indices and cell counter-generated histograms examined to determine the type of anaemia. As can be seen in Table 1, there were 90 cases of dimorphic anaemia, 4 cases of hemolytic anaemia, 40 cases of macrocytic anaemia, 216 cases of microcytic hypochromic anaemia, and 150 cases of normocytic normochromic anaemia (Table 1).

Table 1: Table 1: Distribution of anaemia based on RBC indices and histogram

Type of anaemia	Number of cases	Percentage
Normocytic Normochromic	150	30
Microcytic Hypochromic	216	43.2
Macrocytic	40	8
Dimorphic	90	18
Hemolytic	4	0.8

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In this investigation, it was found that, as per histogram findings, microcytic hypochromic anaemia was the commonest type found in this study, 43.2% of total anemias. Age-wise distribution of anaemia showed that the preschool and reproductive age groups showed mostly microcytic hypochromic anaemia and the elderly age groups showed mostly normocytic normochromic anaemia. The mean MCV values for different anaemias was 83.1 fl for normocytic, 67.7 fl for microcytic, 101.3 fl for macrocytic, 80.1 for dimorphic, and 71.5 fl for haemolytic anaemia. Mean MCH values for different anaemias was 27.5 pg for normocytic, 21.3 pg for microcytic, MCH 37.6 pg for macrocytic, 27.4 pg for dimorphic, and 21.8 for hemolytic.

Histogram patterns revealed that 34 cases out of 100 cases of dimorphic anaemia had left shift curve, while 10 cases of hemolytic anaemia had 5 left shift and 5 broad base curves. 130 out of 153 cases of normocytic anaemia showed normal curve, 148 out of 210 cases of microcytic anaemia showed a left shift curve, and 19 out of 25 cases of macrocytic anaemia displayed a right shift curve.

Among the 500 cases in our analysis, 160 (32%), had a normal curve, 197 cases (39.4%) had a left shift, 33(6.6%) had a right shift, 100 (20%) had a broad base, 2(0.4%) had a bimodal curve, and 8 (1.6%) had a short peak (Figure 1).

Figure 1: Types of Histogram Abnormality

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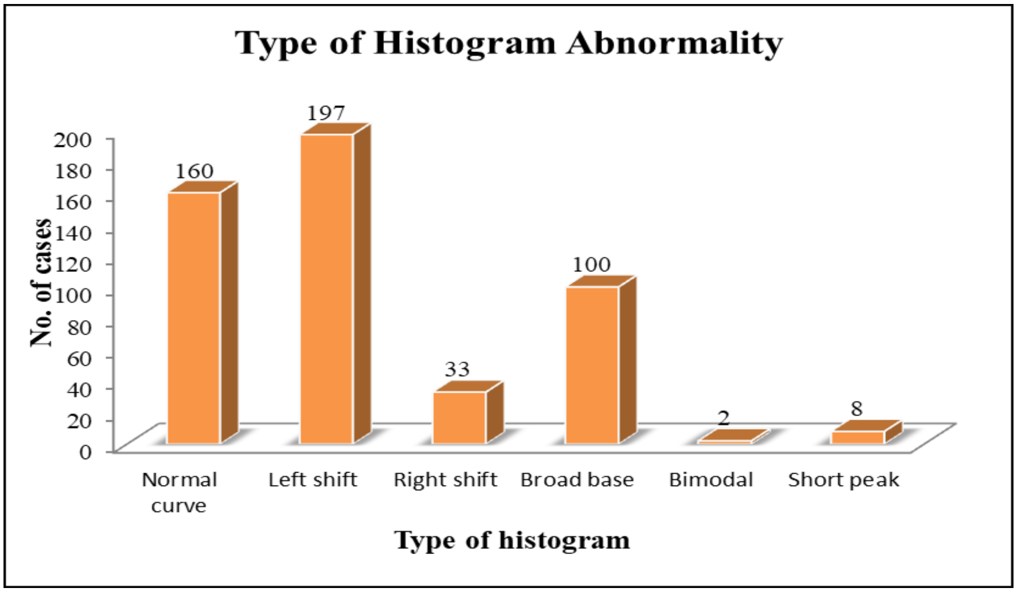

Thirty-one of the 153 cases of normocytic normochromic anaemia in our investigation had a normal curve, and the remaining 23 cases had a broad base curve. Three cases had a short peak. 148 cases had a left shift, 8 cases had a normal curve, 51 cases had a broad base, and 210 cases had microcytic hypochromic anaemia. Of the twenty-five cases of macrocytic anaemia, nineteen had a right shift, two had a normal curve, two had a broad base, and two had a short peak. Of the 100 cases of dimorphic anaemia, 20 had a normal curve, 44 had a left shift, 14 had a right shift, 19 had a broad base curve, 2 had a bimodal curve, and 3 had a short peak. Of the ten hemolytic anaemia cases, 5 had a broad base curve and five had a left shift curve (Table 2).

Table 2: Histogram variations in different Anaemias

Type of anaemia	Normal curve	Left shift	Right shift	Broad base	Bimodal	Short peak
Normocytic	130	-	-	23	-	-
Microcytic	8	148	-	51	-	3

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Macrocytic	2	-	19	2	-	2
Dimorphic	20	44	14	19	2	3
Hemolytic	-	5	-	5	-	-

DISCUSSION

Anaemias has long been a serious health issue, especially for preschool-going children and females who are fertile. A vital component of CBC reporting that aids in providing important information separate from cell counts is microscopic and morphological assessment. The majority of anaemic patients in our current investigation (20.2%) where under the age of ten, a finding that was also observed in the research conducted by Kumar et al.[5] and this was followed by the age group that made up the majority in the investigation performed by Shingla et al.[6] which was those between 21 and 30 (17%). The age group less than 10 years showed male preponderance and the age group of 21-30 years had female preponderance which was also seen in the investigation conducted by Shingla et al.[6] in which out of 63 patients in the age group 1-10 years, 32 were males and 31 were females and in the age group 21-30 years, out of a total of 105 patients, 74 were females and 31 were males. The adult group is currently going through a period of rapid growth and development, which can be used to explain this. Fast growth and frequent blood loss, such as the menstrual cycle, require the body to have more iron in order to function. After the age group 41-50 years, males were seen to be more affected than females and this result was also seen in the studies done by Shingla et al [6] in which the age group 41-50 and above had a total of 198 patients out of which 116 patients were males and 82 patients were females. The total population showed male preponderance which was in discordance with the investigations observed by Rao et al.[7] and Jain

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et al.[8] The increased population of males having anaemia in our study would most likely be due to the exclusion of antenatal care female patients with anaemia. The mean value was computed for each parameter across the various diagnostic groups. 83.1 fl for normocytic, 67.7 fl for microcytic, 101.3 fl for macrocytic, 80.1 for dimorphic and 71.5 fl for haemolytic anaemia were the mean values reported by MCV. According to the investigation conducted by Venkatachalam and Rashmi [9], the mean values for MCV were 87 fl for normocytic, 71.6 fl for microcytic, 99.5 fl for macrocytic, 83.8 fl for dimorphic and 71.9 fl for hemolytic. These values were in agreement with the findings of that study.

The form of the data distribution can be used to interpret a histogram. Red cell populations can be categorized as single or dimorphic (multiple) based on the distribution shape of the histogram. The histogram of a single-population can exhibit any combination of these features, as well as a widened, skewed, bell-shaped, or Gaussian curve. The histogram can be symmetric, bimodal, or bimodal but skewed in dimorphic populations.

A histogram is a graphical representation of cell size and cell number. Usually, the curve has a bell-shaped symmetry and is referred to as a Gaussian distribution. The normal curve will lie between 80-100 fl in the normal MCV range. Curve shifts that are only in one direction are crucial for diagnosis. The number of cells per channel is represented in the Y axis, while the X axis displays cell size. Thus, the curve will move to the right and vice versa as the cell size increase. RBCs are defined as cells having a volume size of 36-360 fl. In our investigation, out of 500 patients, the type of histogram variations showed a majority of left shift curves (197 cases) which were also seen as the majority in studies done by Shingla et al.[6] While the study done by Rao et al.[7] and Swami et al.[10], most cases demonstrated a broad base curve (37.72% and 38.63% of cases respectively).

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The existence of several populations of cells with different sizes- normocytic, microcytic, and macrocytic – can account for the wide base curve. The bimodal red cell histograms are typically related to the response to hematinic agents and/or therapeutic transfusions for microcytic and macrocytic anaemia, but they can also show other causes like folate/vitamin B12 deficiency, megaloblastic anaemia following iron treatment, and early iron deficiency anaemia [6]. Because a dimorphic population can manifest as a mixture of cells of different sizes and forms with or without normal red blood indices, or as a dual population of microcytic and normocytic or normocytic and macrocytic red cells, it is imperative to examine the peripheral smear to look at all cell populations. One risk of obtaining a false diagnosis is depending solely on automated values. Morphological findings, in practice, should be correlated with the graphical and numerical data for better result interpretation mostly because dimorphic blood picture is usually associated with aberrant red cell population

A broad base curve because of the high red cell distribution width represents anisocytosis. A left shift in microcytic anaemia is due to low MCV. A short peak is seen in cases with very low hemoglobin value and a right shift is seen in cases with high MCV.

CONCLUSION

We conclude that, in a busy day-to-day laboratory doing 300+ CBCs per day, where time has become very valuable, reviewing a peripheral smear for every CBC done becomes difficult.

Hence, in many cases, histograms would lead the pathologist/ consultant toward the right diagnosis without the need for making a peripheral smear. A vital diagnostic technique remains the blood smear examination, even in the era of molecular analysis. However, histograms can reveal subtle changes well before numerical parameters and subjective assessment, such as in megaloblastic anaemia and emerging iron deficiency cases.

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