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A Study On RBC Histogram In Different Morphological Types Of Anemia In Comparison With Peripheral Blood Smears In A Tertiary Care Centre In Rural South India.

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ABSTRACT

Erythrocytes are the most abundant and one of the most important cells in circulation in humans. They are biconcave, non-nucleated cells of the size of around 7.4 μm in diameter carrying haemoglobin. Various diseases and physiological conditions alter the morphology and contents of the red blood cells. The study of these alterations could be a major clue to the diagnosis and management of these diseases. Anaemia is one of the most common morbidities associated with altered RBC morphology and content. To study RBC histogram patterns in different types of anaemia (Microcytic hypochromic, Macrocytic Normocytic normochromic) and Comparison of automated RBC parameters in anaemic patients with morphological features noticed on peripheral smear examination. A Hospital-based cross-sectional study, in the Department of Pathology at Government Medical College and Hospital, cuddalore district, Annamalainagar, from November 2023 to December 2024. Patients sent to the haematology lab for evaluation of anaemia were included in the study population with a sample size calculated by the formula $1.96^2 pq/d^2$. Blood samples of patients were sent to the haematology lab for evaluation of anaemia by complete blood count (CBC) and peripheral smear examination. In this present study Of the total 488 anaemia cases studied 286 were females, with a female to male ratio of 1.4:1.There were 121 female patients who belonged to the reproductive age group of 15-49 years. The maximum number of anaemia cases (77.04%) was present in an adult population of more than 15 years of age and the mean age group was 42 years. Majority of the cases (64.58%) showed moderate anaemia, 24.8% cases showed severe anaemia and 10.62% cases showed mild anaemia. Blood samples of 488 patients with anaemia were evaluated along with the corresponding peripheral smear findings. The cases were Normocytic normochromic anaemia, Microcytic hypochromic anaemia, dimorphic anaemia, and Macrocytic anaemia as diagnosed by peripheral smear. In our study, 64.2% of the cases were Normocytic normochromic anaemia and 35.2% of cases were microcytic hypochromic anaemia. Macrocytic anaemia was observed only in 0.4% of cases and dimorphic anaemia only in 0.2 % of the cases as diagnosed by peripheral smear examination. This study also showed that there could be an overlapping correlation of various histograms with different types of anaemia diagnosed morphologically on the peripheral smear.
Keywords: Anaemia, morphology, red blood cell, histogram, smear, haemoglobin

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INTRODUCTION

Anaemia is a major public health issue predominant in children and females of reproductive age groups of low to middle-income populations in developing countries like South Asia. Anaemia constitutes an important diagnostic and clinical category of haematological disorders prevalent all over the world. As of 2010 statistics prevalence of anaemia was approximately 32.9% worldwide resulting in 68.36 million years lived with disability [1]. These changes can be evaluated either by age-tested laborious techniques, or the peripheral blood smear evaluation. However, a recent alternative is the use of an automated haematology analyzer. This assesses all the cells passing through the predetermined chambers and gives a large number of blood cell indices. It works based on Coulter's principle. The analyzer used in this study was Sysmex XS 1000i. Automated haematology analyzer counts all the non-nucleated cells in the range of 25-250 fL as RBCs and gives a corresponding histogram along with all the red blood cell indices. RBC histogram is an integral part of the automated haematology analysis with the size of cells being plotted along the X-axis and several cells being plotted along the Y-axis. The normal histogram is a Gaussian curve. The histogram obtained from the study was compared with a reference curve and each histogram categorized was into a different type [2]. A study of RBC histograms done by Jithendra Chavda *et al* on 500 anaemia cases along with peripheral smear examination concluded that histograms could provide a major hint on RBC disorder and guide in peripheral smear examination along with indices [3]. Another study by Sandhya. I & Muhasim, to assess the utility of RBC histogram along with peripheral smear revealed that histogram should be considered as a screening tool though not confirmatory for any pathological diagnosis [4]. Asuthosh S Kumar *et al* conducted a study on 60 anaemia cases, with the objective of standardization and grading of abnormal blood cell morphology in peripheral blood smear, counter-based red cell indices in case of anaemia of various aetiologies and comparative evaluation of peripheral blood smear examination and automated red cell indices including RDW, MCV, MCH, MCHC [5]. Peripheral smear is considered the standard technique for the morphological evaluation of red blood cells and histograms are always available as an integral part of the automated haematology analysis. So through this study, we intended to evaluate the utility of histograms in narrowing down the differential diagnosis and hence arriving at a rapid diagnosis. Anaemia is a common health issue in developing countries especially among children and females of reproductive age group [7]. Complete blood cell count is a routine blood test performed in the clinical laboratory. It includes white blood cell count, haemoglobin, platelet count, and red blood cell indices [8]. Complete blood cell count is currently done using an automated haematology analyzer and RBC histograms are an integral part of automated haematology analysis. While the majority of clinicians and laboratory personnel are aware of the utility of RBC indices in the categorization of anaemia, the significance of RBC histograms is being underestimated in the evaluation of anaemia. Automated haematology analyzer counts all the cells present in the sample passing through the predetermined chambers and hence the RBC histogram produced by the assessment of the larger number of cells is likely to have a higher power of study. Presence of multiple populations of RBCs is more likely to be picked up by the histograms than the indices which are more of mathematical value. RBC histogram being a pictorial depiction of data can convey the message of the data easier even to a non-pathologist [4]. All these efforts to classify anaemia are to detect easily rectifiable causes such as nutritional deficiencies, and anaemia demanding urgent attention such as haemolysis, and anaemia of renal insufficiency, and bring them to the attention of the physician or the pathologist. This helps them to narrow down their differential diagnosis and hence reduce the battery of tests required for patient management [6].In this study, we attempt to classify histogram patterns into an increased number of subcategories and compare them with the peripheral smear findings. The conventional method of categorization of histograms will also be compared with the peripheral smear. RBC indices will also be correlated with the corresponding peripheral smear findings. Thus we intend to make an analysis of RBC histogram more user-friendly and to help the clinician narrow down the differentials even before peripheral smear confirmation by a pathologist. We also have analyzed the pattern of anaemia and its distribution among patients who have come to the hospital, which may be a representation of the anaemia pattern of the rural area.

MATERIALS AND METHODS

A Hospital-based cross-sectional study, in the Department of Pathology at government medical college and & Hospital, Cuddalore, from November 2023 to December 2024. Patients sent to the haematology lab for evaluation of anaemia were included in the study population with a sample size calculated by the formula $1.96^2pq/d^2$. Blood samples of patients were sent to the haematology lab for evaluation of anaemia by complete blood count (CBC) and peripheral smear examination. Consecutive

samples of anaemia diagnosed with a haemoglobin level lower than the normal limits for the age and sex of the individual. All the study samples were analyzed in the automated haematology analyzer SYSMEX-XS 1000i and a peripheral smear was prepared.

Inclusion Criteria

- Blood samples of all anaemic patients presenting to Department Of Pathology, Government Medical College and Hospital, Cuddalore district, Annamalainagar, central laboratory with haemoglobin percentage below the lower limit of normal will be included in the study.
- Patients of all age groups will be included in the study.

Exclusion Criteria

- Incomplete request forms.
- Samples for which both CBC and peripheral blood smears are not requested.

Data Analysis

- Data was entered in Microsoft Excel and analysis was done with the help of IBM SPSS version 25 software.
- The proportion of various patterns of anaemia was expressed as a percentage with a 95% confidence interval.
- The percentage was calculated to look for a comparison between different types of anaemia diagnosed through peripheral smear examination.
- The Pearson correlation coefficient was calculated to assess the correlation between RBC parameters and findings on peripheral smears.
- The confidence interval of 95% will be taken as statistical significance.

RESULTS

Demographic Details of Study Population

Table 1: Gender Distribution of Anaemia

Gender	Number	Percentage
Male	202	41
Female	286	59
Total	488	100

Table 2: Age Distribution of Anaemia

Age	Frequency	Percentage %
Birth	2	0.410
One Month	2	0.410
2-6 Month	4	0.820
6 Months-6 Years	83	17.008
6-14 Years	21	4.303
>15 Years	376	77.049
Total	488	100

Table 3: Grading Severity of Anaemia

Severity of anaemia	Frequency	Percentage
Mild	51	10.62
Moderate	310	64.58
Severe	119	24.8
Total	480	100

Table 4: Pattern of anaemia in the study population

Type of Anaemia	Frequency	Percentage
Dimorphic Anaemia	1	0.2
Macrocytic Anaemia	2	0.4
Microcytic Hypochromic Anaemia	172	35.2
Normocytic Normochromic Anaemia	313	64.2
Total	488	100.0

Table 5: Categorization of morphological type of anaemia concerning severity

Table 5A: Mild Anaemia

Peripheral SMEAR	Mild Frequency	Anaemia Percentage
MHA	17	33.33
NoNc	33	64.70
Macrocytic	-	-
Dimorphic	1	1.9
Total	51	100

Table 5b: Moderate Anaemia

Peripheral SMEAR	Moderate Frequency	Anaemia Percentage
MHA	83	26.77
NoNc	227	73.22
Macrocytic	-	-
Dimorphic	-	-
Total	310	100

Table 5c: Severe Anaemia

Peripheral Smear	Severe Frequency	Anemia Percentage
MHA	70	58.8
NoNc	47	39.5
Macrocytic	2	1.7
Dimorphic	-	-
Total	119	100

Table 6: Histogram Patterns (95% C.I)

Type of histogram	Frequency	Percentage
Left Shift	161	33
Normal	126	25.8
Normal (Short Peak)	113	23.1
Left Shift (Broad Base)	38	7.8
Left Shift (Short Peak)	34	7
Normal (Broad Base)	7	1.4
Right Shift (Short Peak)	5	1
Double Peak	4	0.8
Total	488	100

DISCUSSION

Anaemia is a major problem affecting all age groups across the world. Anaemia is a clinical condition in which there is decreased red blood cell count and haemoglobin levels [8]. Anaemia is prevalent in about one-fourth population of the world. Anaemia plays an important role in morbidity and mortality among children. Anaemia is a manifestation and not a disease per se. Malnutrition is the most common cause of anaemia among children. Nutritional anaemia due to iron deficiency, vitamin B12 or folic acid deficiency, and hookworm infestation can be easily prevented and treated [9]. Routine investigations like complete blood counts and Peripheral smears are useful to investigate cases of anaemia [10]. Of the total 488 anaemia cases studied 286 were females, with a female-to-male ratio of 1.4:1. There was 121 female patients who belonged to the reproductive age group of 15-49 years. The age distribution of anaemia patients in the current study was as follows Maximum number of anaemia cases (77.04%) was present in adult population of more than 15 years of age and the mean age group was 42 years. The majority of the cases (64.58%) showed moderate anaemia, 24.8% cases showed severe anaemia and 10.62% cases showed mild anaemia. In this study, blood samples of 488 patients with anaemia were evaluated along with the corresponding peripheral smear findings [11]. The cases were Normocytic normochromic anaemia, Microcytic hypochromic anaemia, dimorphic anaemia and Macrocytic anaemia as diagnosed by peripheral smear. In our study, 64.2% of the cases were Normocytic normochromic anaemia and 35.2% of cases were microcytic hypochromic anaemia. Macrocytic anaemia was observed only in 0.4% of cases and dimorphic anaemia only in 0.2 % of the cases as diagnosed by peripheral smear examination [12]. The majority of the subjects of Normocytic normochromic anaemia had (227 cases) a moderate degree of anaemia and 47 had severe anaemia followed by mild anaemia seen in 33 subjects. Of the 172 Microcytic hypochromic anaemia cases majority i.e.83 cases had a moderate degree of anaemia 17 had mild anaemia. Both the 2 cases of Macrocytic anaemia were severe in grade and the single case of dimorphic anaemia was mild in degree. This categorization did not include children less than 6 months of age Of a total of 488 cases studied, the majority of the histograms showed a left shift curve in 161 (33%) cases, followed by the normal curve in 126 (25.8%) cases [13]. Other histograms patterns observed less frequently were Normal short peak curve in 113 (23.1%) cases, Left shift curve with a broad base in 38 (7.8%) cases, Left shift curve with a short peak in 34 (7%) cases, Normal curve with a broad base in 7 (1.4%) cases, Right shift curve with short peak in 5 (1%) cases and bimodal curve in 4 (0.8%) cases [14,15].

CONCLUSION

RBC histogram is an automated analyzer-generated graphical representation of RBCs with size being plotted on the X-axis and number being plotted along the Y-axis. The study showed that the sub-categorization of histogram patterns did not generate any additional information from the histogram than obtained from the broad categorization of the Left shift curve, Right shift curve, and Normal curve. This could probably be sample bias and hence require future studies. This study also showed that there could overlapping correlation of various histograms with different types of anaemia diagnosed morphologically on the peripheral smear. This could be due to causes that may not be picked up by the examination of peripheral smear alone, like the presence of a small number of fragmented RBCs may give the impression of Normocytic normochromic anaemia on peripheral smear but the histogram may give left shift curve. The study also showed that a short peak of the histogram is suggestive of a reduced RBC population. Hence, clues given by the RBC histogram should be thoroughly analyzed by the pathologist before the evaluation of the peripheral smear examination.

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