



Study of RBC Histograms and Its Correlation with Etiopathogenesis and Other Parameters in Various Anemias

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The pluripotent stem cells which are present in the bone marrow renew by its own and differentiate into mature cells. These stem cells undergo division by the presence of erythropoietin, where the nucleus is extruded out from the cell during the end of differentiation, thereby retaining cytoplasmic RNA to form a reticulocyte. The reticulocyte is a precursor to red blood cell and on losing the RNA it matures into a Red Blood Cell. The present study aimed to analyze the correlation between the automated histogram patterns along with morphological features of RBC's prepared from peripheral smear examination in different types of anemia. viz., MCV, MCH, MCHC & RWD- CV.

Keywords: Histograms; etiopathogenesis; MCV; MCH; MCHC.

1. INTRODUCTION

Anemia is a condition in which the physiological function have not been supplied with adequate

oxygen due to the reduced number of blood red blood cells (RBC) or any hindrances in their oxygen carrying capacity. It may vary with a number of factors including age, sex, altitude,

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smoking, and pregnancy status. Iron deficiency is the predominant cause of anemia occurring globally besides other important conditions particularly deficiencies in folate, vitamin A and B12 synthesis. Apart from these, chronic inflammation, parasitic infections, and inherited disorders can also cause severe anemia in people. Complete blood count (CBC) using automated analyser and microscopic peripheral smear examination are the main diagnosis methods. Complete blood count (CBC) is a routine test conducted nowadays to evaluate the concentration & count of various cellular components of blood such as RBC count, WBC count, platelet count, Hb, haematocrit, red cell indices, differential count (WBC), Mean platelet volume, histograms of RBC, WBC, platelets & red cell distribution width. In recent days, many of the laboratories have replaced the traditional methods in haematology by automated analysers & automated data [1]. As there is advancement in the technology of automated analyzers with increase in its precision, the rate of manual peripheral smear review have been declining [2]. RBC histogram is a symmetrical bell-shaped curve, a diagrammatic representation for better understanding & interpretation of various anemias. But it is still limited in day to day use as the technologists are unaware & only a few have understanding in correlating & interpreting [1]. Width of red blood cells, haemoglobin distribution and its width and reticulocyte cell count are the parameters that have gained popularity as they provide useful information & a less importance is given to scatter plots & histograms [3,4,5]. In spite of the latest sophisticated instruments present today, there are certain manual techniques that we still rely upon. Our study was planned & conducted with an aim to observe the relationship between BC- 5380 Mindray Auto Haematology Analyser and peripheral smear analysis, using human blood samples in the department of Pathology, Sree Balaji Medical College & hospital, Chennai.

2. MATERIALS AND METHODS

This is a prospective study conducted in the department of pathology, Sree Balaji Medical College and Hospital after getting Institutional Ethical Committee approval. Patients were sourced from the clinical departments. A total of 500 patients with anemia were studied for 24 months since October 2015 to September 2017.

2.1 Inclusion Criteria

Patients presenting with anemia with hemoglobin levels, less than 11.5 gm%, were included for the study irrespective of patients age group.

2.2 Exclusion Criteria

Patients with a recent history of blood transfusion were excluded from the study. Samples with inadequate quantity (< 3 ml) for analysis and study were excluded.

2.3 Method of Sample Collection

A 3 ml of venous blood sample is collected in a EDTA BD Vacutainer, from all the patients that were included in the case, & was used for Automated analysis & peripheral smear. Study. The automated analysis was done using BC-5380 Mindray Auto Hematology Analyzer. It is a fully automatic 5-part differentiation of WBC with 27 parameters, 3 histograms & 1 scatter gram. It uses laser scatter, advanced flow cytometry & chemical dye methods. Peripheral smear study was done in all the cases simultaneously. The peripheral smear reporting was done without privy to the histogram pattern of the concerned sample.

2.4 Staining of Thin Blood Smear

Leishmans stain was used in this study for the staining of blood smear. Leishmans stain is added drop by drop to the slide and it is left to wait over a period of two minutes, which helps in fixation of the blood smear. Double the quantity of buffered / distilled water is added to the slide and mixed gently for 8 minutes. It is washed in a slow stream of running tap water and left to dry. After drying, the slide is studied under oil immersion lens of the microscope [6-9].

2.5 Statistical Data Analysis

The data collected in the study were assorted using Microsoft excel and analysis of the data was done using IBMSPSS statistics software, Pearson's Chi square test was used wherever appropriate.

3. RESULTS

This present prospective study was carried out in the department of Pathology, Sree Balaji Medical College & Hospital for 24 months duration started

from October 2015 to September 2017. A total of 500 cases of anemia that belonged to the inclusion criteria were studied.

3.1 Age of the Study Group

The study comprised of a population with the age group ranging from 1 to 93 years. Major cases of patients were between 41 to 50 years of age.

3.2 Sex Distribution

The majority of the study population were females which contributed 61% of the total.

3.3 Classification of Anaemia Based on Haemoglobin

Based on the levels of haemoglobin, anaemia is classified as Mild, Moderate & Severe.

- Mild > 10.0 gm/ dl
- Moderate, between the values of 7.1 and 9.9 gm/ dl
- Severe, < 7 gm/ dl

3.3.1 Distribution of cases based on peripheral smear study

Among 500 cases which comprise the whole of the study population, peripheral smear study

was done for all the cases and based on the interpretation; the distribution of various anaemia was analyzed. Diagnosis based on morphology alone was carried. Cases that had predominantly microcytic hypochromic RBCs were reported as Microcytic Hypochromic anaemia, & those with macrocytic RBCs were reported as Macrocytic anaemia. Cases which had multiple populations of cells, like microcytic hypochromic RBCs with Macrocytic RBCs & Normocytic Normochromic RBCs were reported as dimorphic anaemia. Some cases had a major population of normocytic normochromic RBCs with or without normocytes or macrocytes; they were reported as Normocytic Normochromic anaemia. Out of 500 cases 361 (72.2%) cases were Microcytic Hypochromic anemia with a majority of 233 female cases. 23 cases were reported as Macrocytic anaemia. Dimorphic anemia was interpreted in 97 cases & Normocytic Normochromic Anaemia was reported in 19 cases [10-24].

3.3.2 Anemia based on RBC histogram pattern

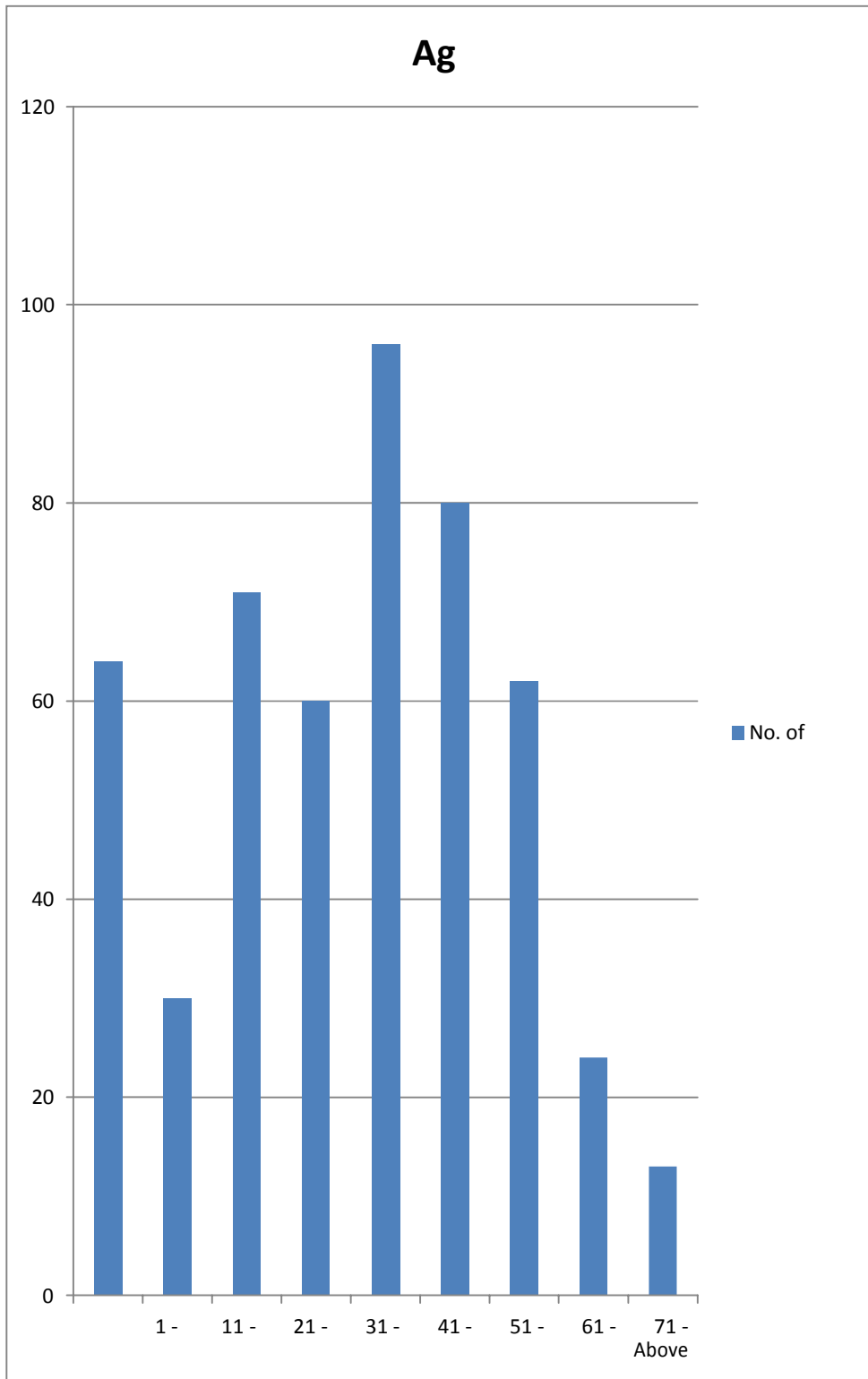
Histogram patterns of all 500 cases were studied and among this

Table 1. Age variation of the study population

		Age Groups			
		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	1 - 10	64	12.8	12.8	12.8
	11 - 20	30	6.0	6.0	18.8
	21 - 30	71	14.2	14.2	33.0
	31 - 40	60	12.0	12.0	45.0
	41 - 50	96	19.2	19.2	64.2
	51 - 60	80	16.0	16.0	80.2
	61 - 70	62	12.4	12.4	92.6
	71 - 80	24	4.8	4.8	97.4
	81 and Above	13	2.6	2.6	100.0
	Total	500	100.0	100.0	

Table 2. Correlation of peripheral smear & RBC histogram pattern

Peripheral Smear	Histogram Pattern					Total
	BB	BM	LS	NC	RS	
Microcytic Hypochromic Anaemia	8	12	317	24	0	361
Macrocytic Anaemia	20	0	0	0	3	23
Dimorphic Anaemia	36	16	24	21	0	97
Normocytic Normochromic	0	0	3	16	0	19
Total	64	28	344	61	3	500



Graph 1. Distribution of age among the study population

344 (68.8%) cases had Leftshift curve, 3 (0.6%) had Right shift curve, 64 (12.8%) cases had Broad base curve, 28 (5.6%) cases had bimodal curve, 61 (12%) had normal curve.

3.3.3 Correlation of RBC histogram patterns with peripheral smear study

The histogram patterns obtained for each case is correlated with the peripheral smear and tabulated for statistical analysis.

3.3.4 Microcytic hypochromic anaemia

Among the cases which were reported as Microcytic Hypochromic Anemia 87.8% cases showed Left Shift in the histogram pattern & 6.6% showed normal curve .

3.3.5 Macrocytic anaemia

Among the cases which were reported as Macrocytic Anaemia 86.9% cases showed Broad Base curve in the histogram pattern & 13.1% showed Right shift curve.

3.3.6 Dimorphic anaemia

Among the cases which were reported as Dimorphic Anaemia 37.2% cases showed Broad Base curve in the histogram pattern & 24.7% showed Left shift curve.

3.3.7 Normocytic normochromic anaemia

Among the cases which were reported as Normocytic Normochromic Anaemia 84.3% cases showed Normal curve in the histogram pattern & 15.7% showed Left shift curve.

3.3.8 Prevalence of anemia based on Mean Corpuscular Volume (MCV)

In all the 500 samples the MCV values were recorded, & based on the values 57.6% cases showed an MCV value less than 80 f L, 36.6% showed a normal MCV range & only 5.8% case showed MCV > 100 f L.

On applying Pearson correlation between MCH of various anaemia & other parameters it shows the following interpretation. MCH in samples categorized under Microcytic Hypochromic anemia is showing a highly significant correlation ($p < 0.005$) to all the parameters except hematocrit. RDW- CV alone is showing a significant difference in correlation to MCH of cases in Macrocytic Anaemia. MCH in Dimorphic anemia on correlation is showing a significant difference to haemoglobin & haematocrit. MCH of cases with Normocytic Normochromic Anaemia show a significant correlation to RBC count, haematocrit & MCV [25-41].

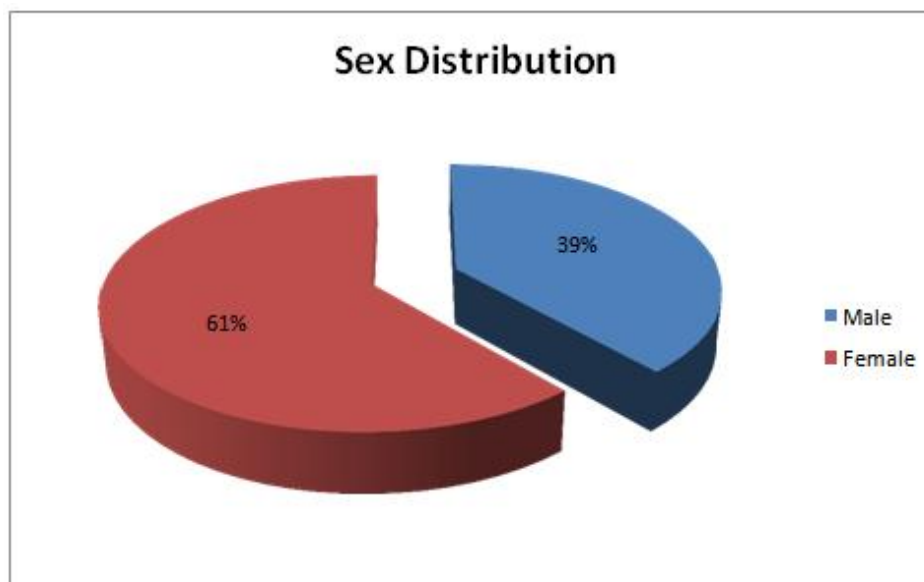
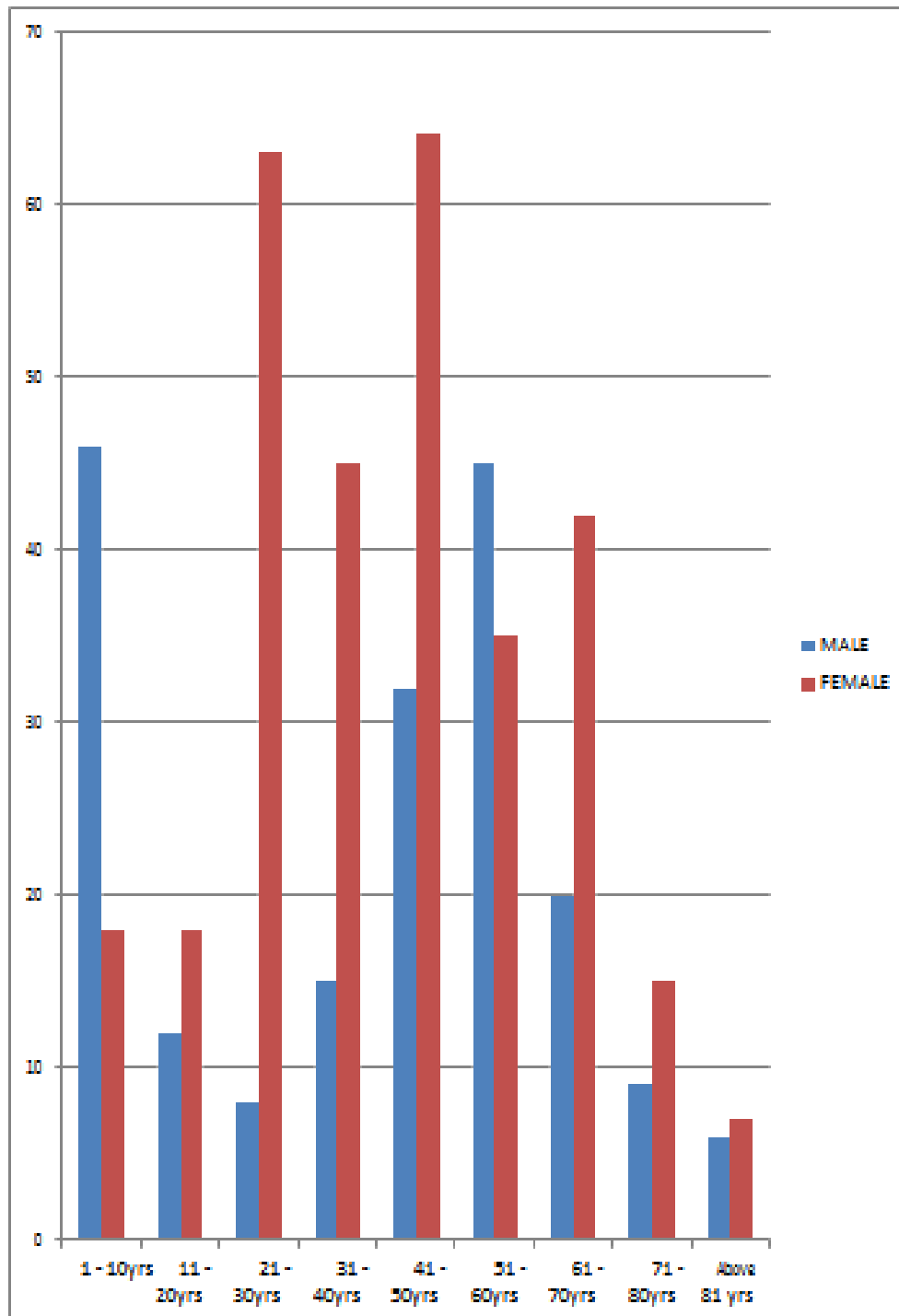


Chart 1. Distribution of sex among the study population



Graph 2. Age and gender wise distribution

Distribution of Anemia based on Mean Corpuscular Haemoglobin Concentration (MCHC): In all the 500 samples the MCHC values were recorded, & based on the values

60.8% cases showed an MCHC value less than 31 gm/ dl, 38.6% showed a normal MCHC range & only 30.6% case showed MCHC > 36 pg.

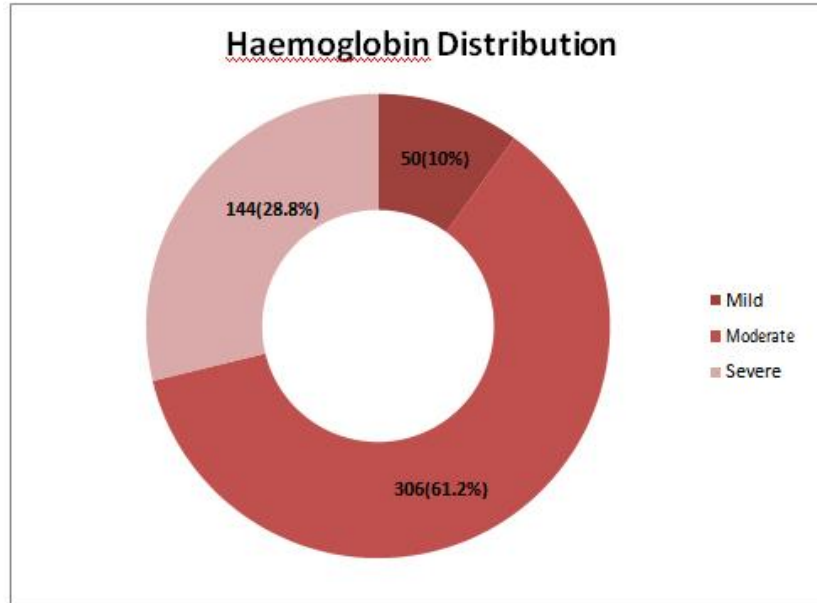
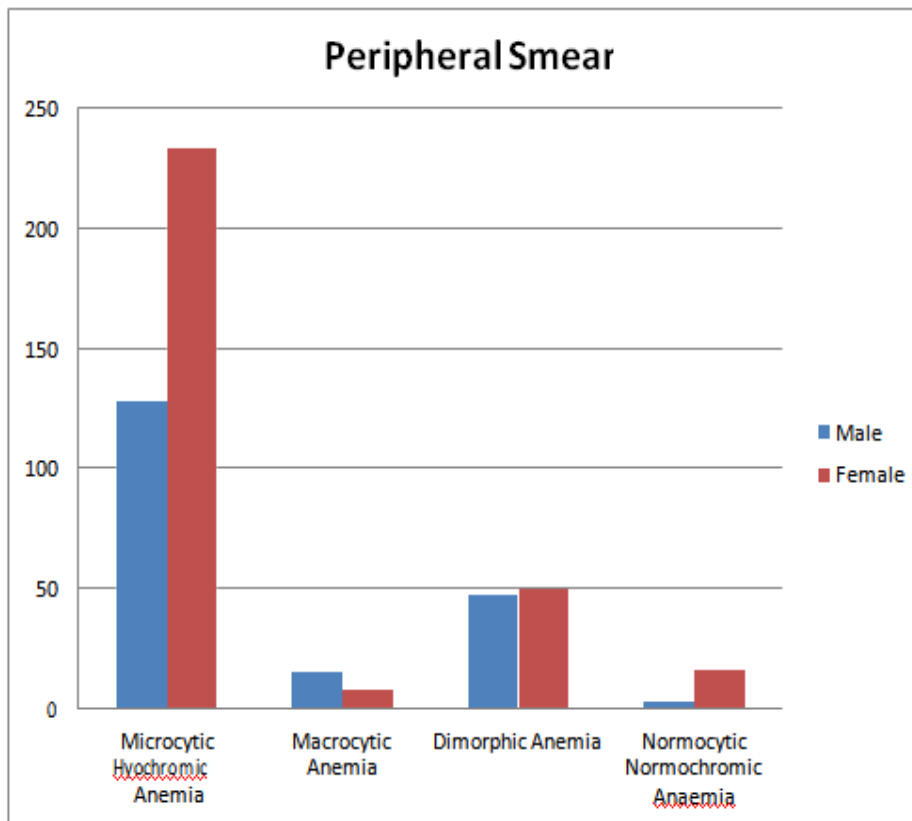


Chart 2. Distribution based on Haemoglobin



Graph 3. Distribution based on peripheral smear Study (gender wise)

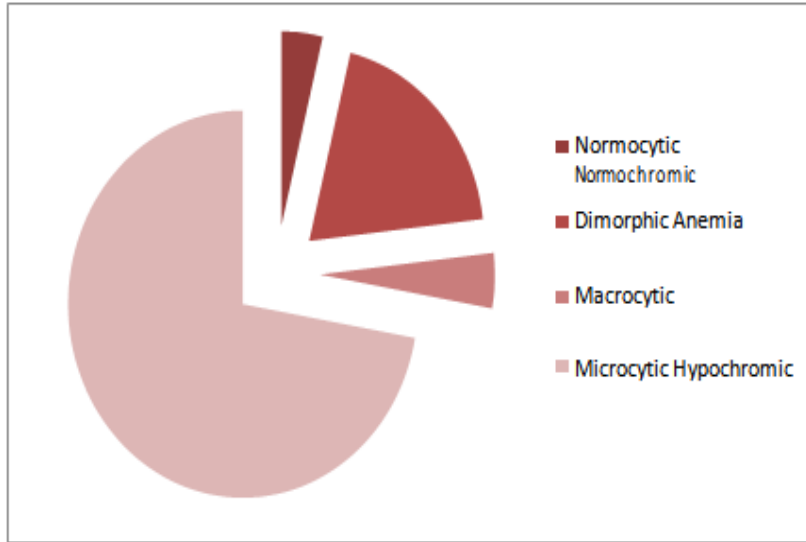
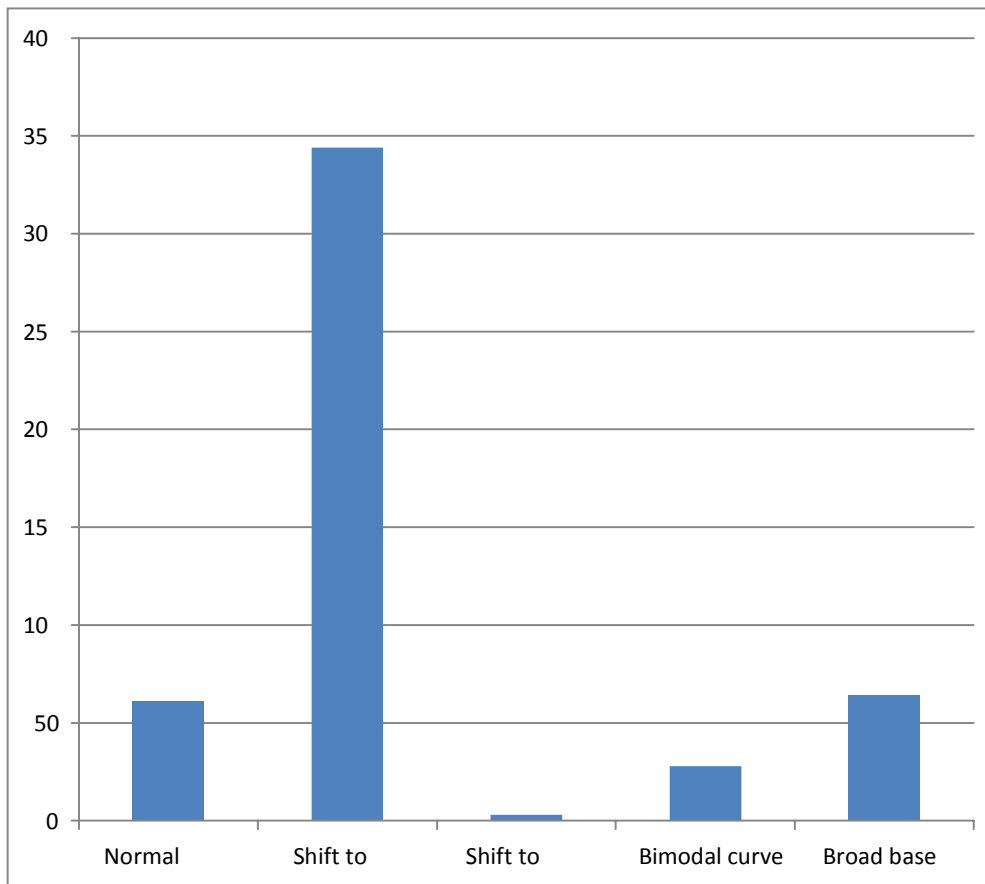


Chart 3. Distribution of anemia based on peripheral smear study



Graph 4. RBC Histogram Pattern wise distribution

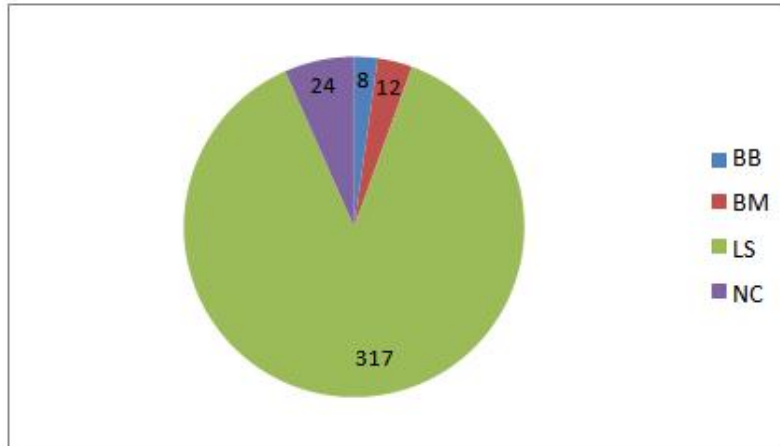


Chart 4. Distribution of histogram patterns in microcytic hypochromic anaemia

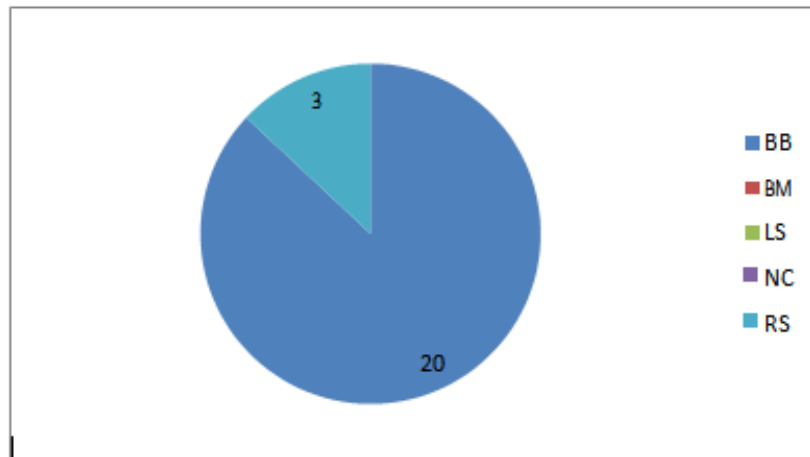


Chart 5. Distribution of histogram patterns in macrocytic anaemia

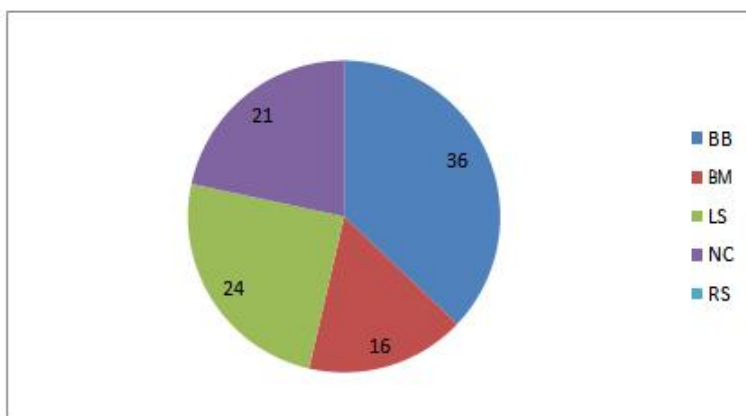


Chart 6. Distribution of histogram patterns in dimorphic anaemia

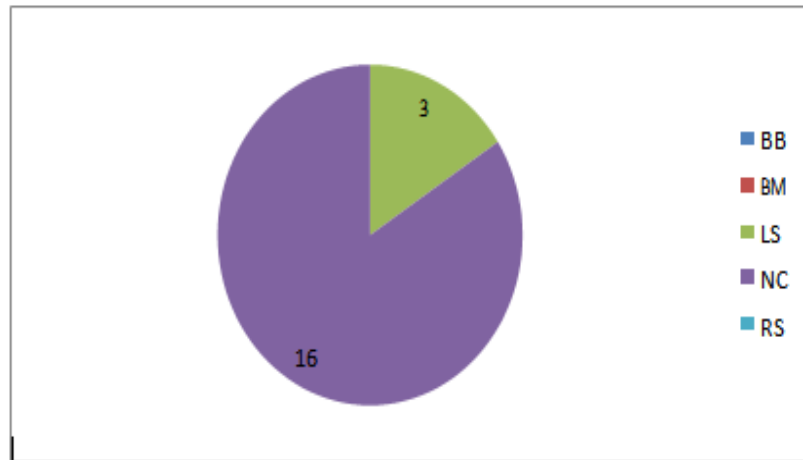
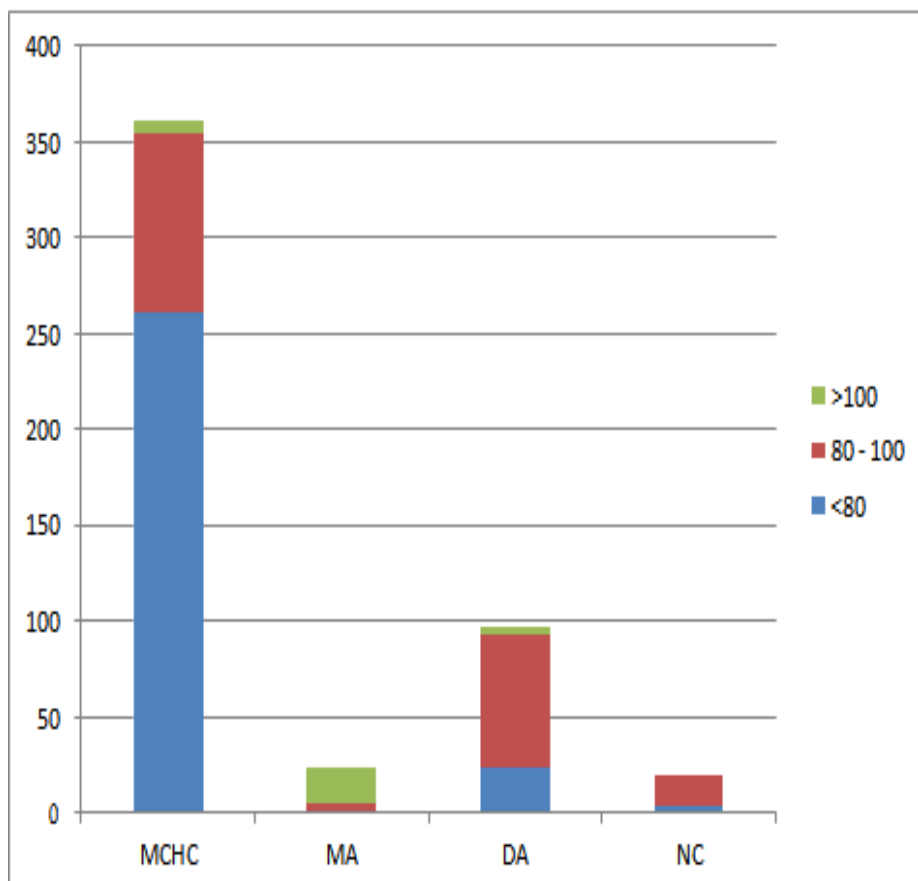
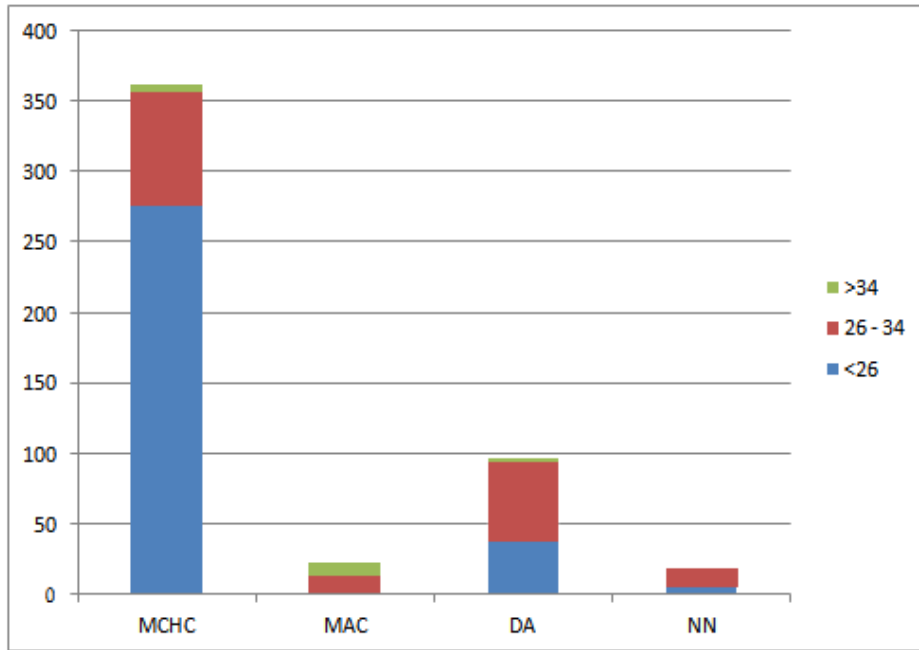


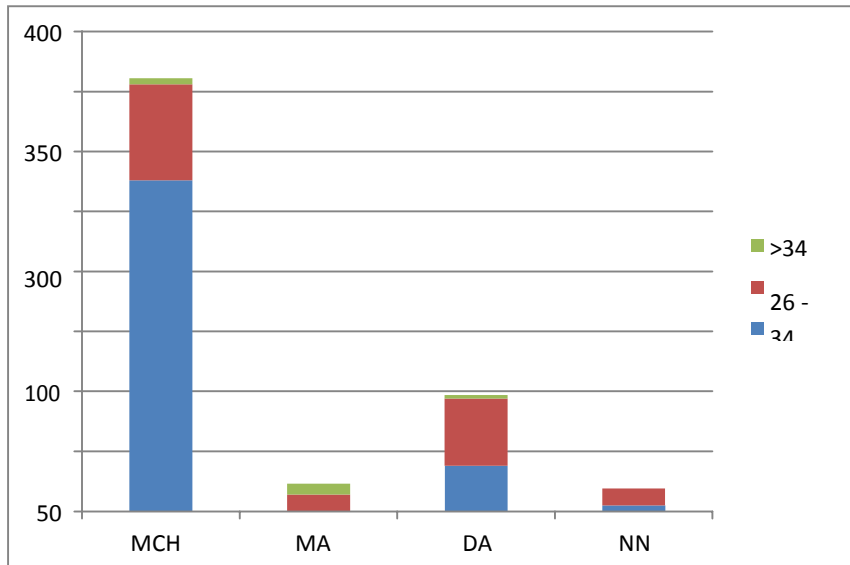
Chart 7. Distribution of histogram patterns in normocytic normochromic anaemia



Graph 5. Distribution of anemia based on MCV



Graph 6. Distribution of anemia based on MCH



Graph 7. Distribution of Anemia based on MCHC

Distribution of Anemia based on Mean Corpuscular Haemoglobin Concentration (MCHC): In all the 500 samples the MCHC values were recorded, & based on the values 60.8% cases showed an MCHC value less than 31 gm/ dl, 38.6% showed a normal MCHC range & only 30.6% case showed MCHC > 36 pg.

In dimorphic anemia the peripheral smear usually shows a dual population of microcytes and normocytes; macrocytes and normocytes; sometimes a mixture microcytes, normocytes and macrocytes. The histogram pattern varies showing a change in the centre of the curve and the width of the curve. The red cell indices may

or may not be normal. Hence diagnosis purely based on automated parameters will be sometimes misleading in this condition. Thus peripheral smear study of the morphology of red cells in correlation will help in interpreting the appropriate diagnosis. Earlier a similar finding was done was they have stated that dual population of RBCs can be identified by histograms in most cases.

3.3.9 Comparative analysis of various anemia's represented by peripheral smear with red cell indices

In all the 500 cases of our study, red cell indices (MCV, MCH, MCHC) was recorded from the automated analyser BC- 5380 Mind ray which also provided the histogram. The red cell parameters were compared and correlated with the peripheral smear findings of each sample. The values of MCV were categorized as microcytic, normocytic/ dimorphic, macrocytic. Cases with the MCV value < 80 f l were labeled as microcytic. Cases that were in the normal range of MCV (8 to 100 f l) were labeled as normocytic or dimorphic. Other cases which had an MCV above 100 f l were labeled as macrocytic. A similar method of labeling was applied for the MCH values of all the cases. Microcytic - < 26 pg; normocytic/ dimorphic - 26 to 34 pg; macrocytic - > 34 pg.

On applying statistical analysis and correlation with two variables one was anemia based on peripheral smear and the other was anemia

based on red cell indices. We obtained P values in the correlations in which some showed significant correlation and a few showed a significant difference. Made a study in which they stated that the MCV level was normal in 61% of anaemic patients among their study population. In our study 36.6% of the total 500 anemia cases had a normal MCV. This shows that MCV alone cannot be used as an independent sensitive parameter to classify anemia. This was studied and they proposed few probable reasons stating that MCV is a mean value and it does not represent the various red cell population present in the blood sample. MCV is insensitive when the microcytes and macrocytes are very few in number. MCH and MCHC also gives a very little information.

This concludes that all cases with decreased haemoglobin levels require a peripheral smear examination for diagnosis. In 2005 Barbara J. Bain [42] is stated that in the current age of automation and even during the age of molecular analysis peripheral smear examination will remain as the most important diagnostic method and along with the latest modern investigative methods peripheral smear examination will also be in light. In comparison to peripheral blood smear examination visual examination of RBC histograms is usually more sensitive and objective in identifying the presence of group of cells that are few in population and morphologically varied in size

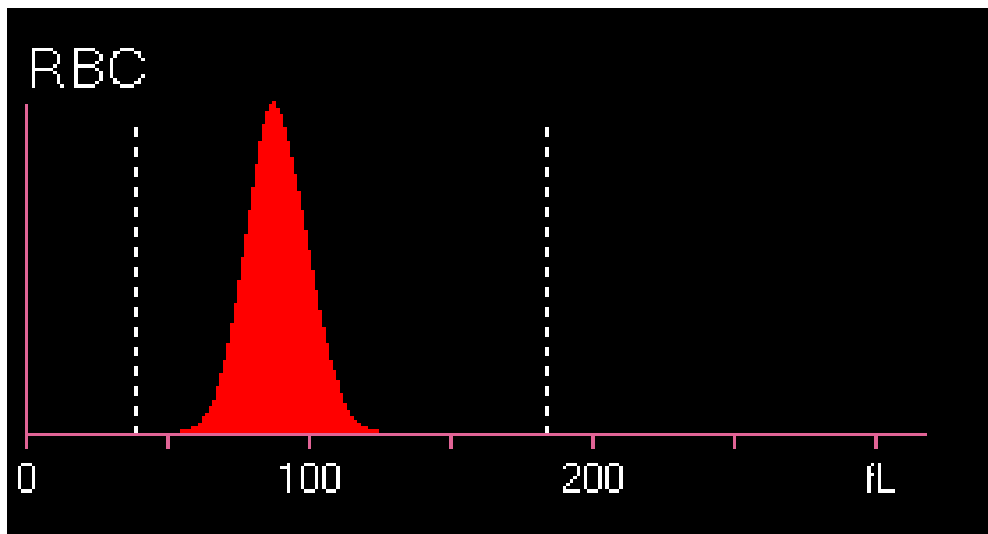


Fig. 1. Normal curve - normocytic normochromic anaemia

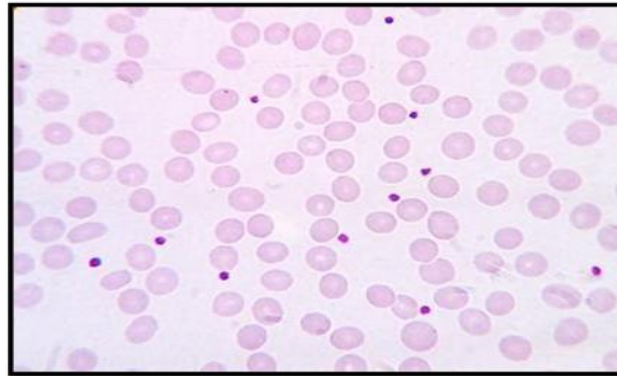


Fig. 2. Peripheral smear- normocytic normochromic anaemia (Leishman stain – 1000X)

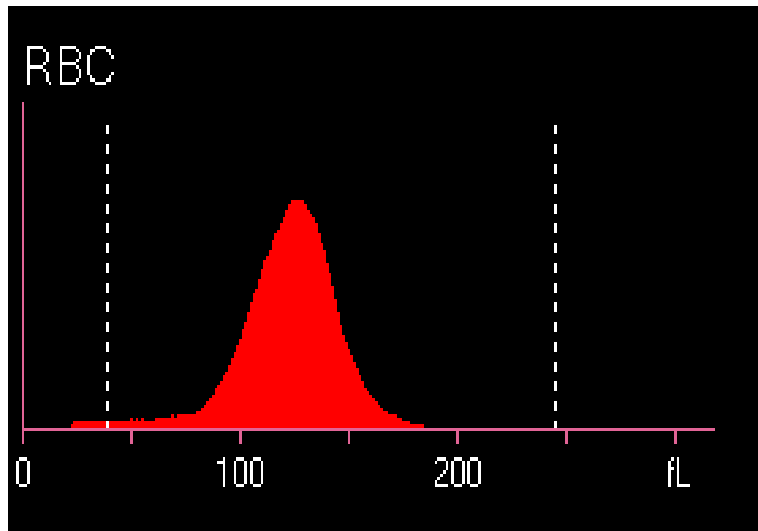


Fig. 3. Right shift histogram - Macrocytic anaemia

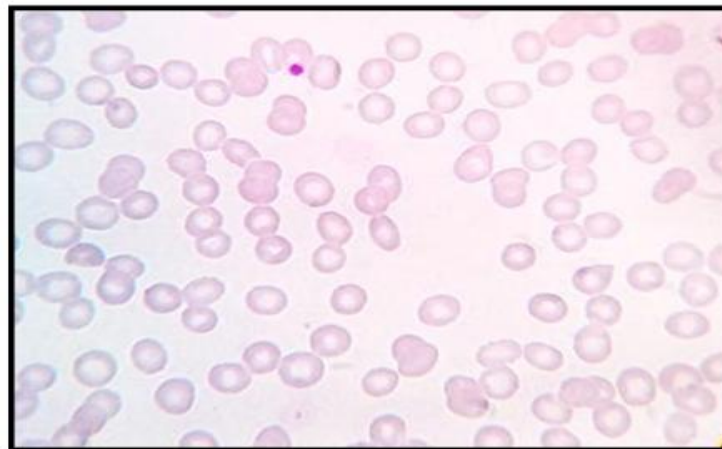


Fig. 4. Peripheral Smear - Macrocytic anaemia (leishman stain – 1000x)

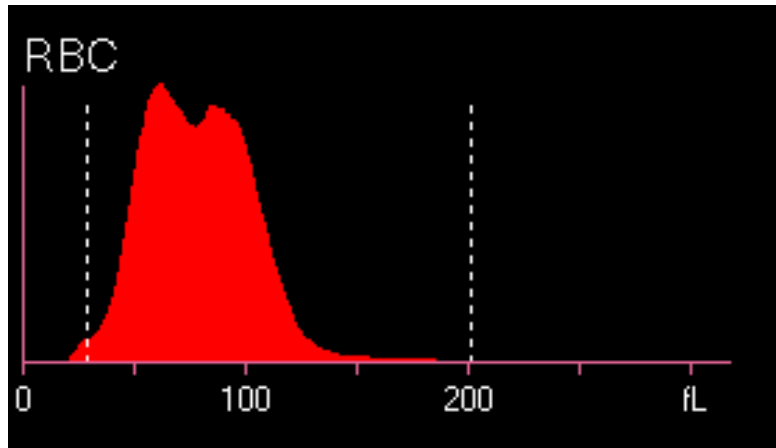


Fig. 5. Bimodal histogram - Dimorphic anaemia

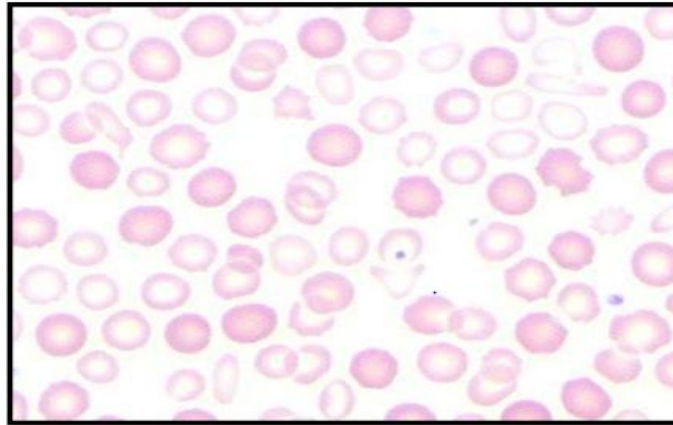


Fig. 6. Peripheral smear - Dimorphic anaemia (Leishman stain – 1000X)

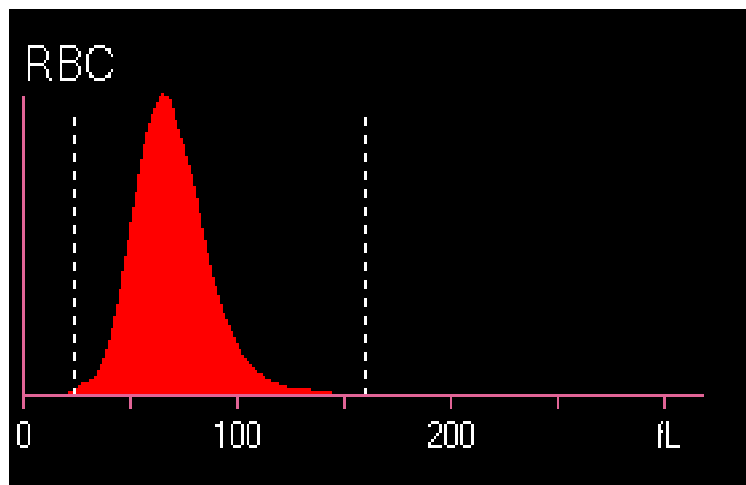


Fig. 7. Left shift histogram - Microcytic anaemia

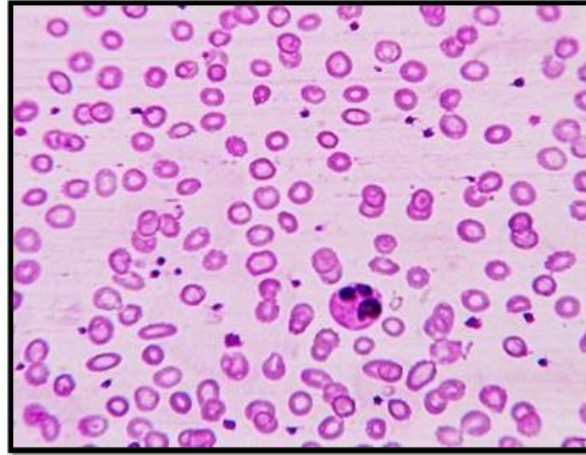


Fig. 8. Peripheral smear - Microcytic hypochromic anaemia (Leishman stain – 1000X)

3.3.10 Red cell distribution width - a parameter for interpretation in anemia

RDW is an additional parameter provided by the automated haematology analysers nowadays. In our study majority of the cases were microcytic hypochromic anemias by peripheral smear study. Among these cases majority had a high RDW. Anisocytosis usually gives a high RDW value. Thus RDW helps in identifying anisocytosis in conditions where the MCV is not in a normal range, like Early Iron Deficiency Anaemia where diagnosis is difficult. In this study, previously we have discussed that cases interpreted as dimorphic anemia had a majority of broad base histograms which explained the presence of multiple populations of RBC and this is described as a high rate of anisocytosis which will automatically reflect as an increase in RDW. It showed that the RDW was increased in case of microcytosis; stated that when there is a higher degree of anisopoikilocytosis there is an increase in RDW. These studies are in concordance with our study.

4. CONCLUSION

The RBC histogram obtained from the automated haematology analyser provides valuable information in view to the diagnosis of various anemias. Only a few studies have been done on RBC histograms to reveal its importance whereas, much more importance was given to WBC histograms to bring it to light for the diagnosis of leukaemia and blast cell population. Our study was done in a purpose to identify the significance of correlation between RBC

histograms and peripheral smear studies in various anemias such as microcytic hypochromic type, macrocytic type, dimorphic type and normocytic normochromic anemia type. The analysis and results showed that there is a significant correlation. However, there is a complex histogram pattern distribution in dimorphic anemia that makes the histogram pattern analysis a non-independent parameter; peripheral smear must be done in such cases as they are in good correlation with histograms. This concludes that peripheral smear examination is an important diagnostic tool even in the period of molecular and automated analysis. As a supplementary to peripheral smear examination RBC histogram and other criteria such as MCV, MCH, MCHC and RDW can be used in diagnosis of various anemias.

CONSENT

As per international standard or university standard, patients' written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

The study was approved by the Institutional Ethics Committee of Sree Balaji Medical College and Hospital.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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