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Utility of red blood cell histograms in various types of anemias in pediatric age group (1-18 years)

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Abstract

Background: Over the past few years, an automated haematology analyzer's complete blood count and a microscopic examination of a peripheral smear have complimented each other to produce a thorough report on patients' blood samples. When a simultaneous peripheral smear is recorded, it is frequently seen that histogram patterns exhibit a variety of properties. Additionally, it is evident that manual reporting of peripheral smears has a number of drawbacks, such as being subjective, time-consuming, and statistically erroneous. Microscopical peripheral smear examination does, nevertheless, also have its benefits.

Aims and Objectives: 1. Utility of red cell histograms in various types of anaemia in pediatric age group (1-18years).

2. To correlate peripheral smear findings with RBC histogram patterns.

Materials and Methods: The present study was undertaken inJNMC and KLE'S DR. PRABHAKAR KORE HOSPITAL and MRC. Hb levels were determined in EDTA sample by the 5 part differential autoanalyzer Sysmex XN 350. Peripheral blood smear of patients having lower limit of the normal range for the particular age and sex were made by push or wedge technique and Leishman staining is done (Procedure-Annexure). Then the slide is studied under microscope and provisional impression is made without referring to the histogram. The RBC histogram generated by the impedance principle and the RBC indices - MCV, MCHC, RDW-CV/RDW-SD were analysed. The impression derived by periprheral smear examination were then correlated with histogram patterns and RBC indices.

Results: In the present study normocytic normochromic anemia was the most common anemia followed by microcytic hypochromic anemia both according to RBC indices and histogram analysis and on peripheral smear. The histogram and indices analysis correlated with peripheral smear findings in majority of microcytic hypochromic anemia, normocytic normochromic anemia and macrocytic anemia. Histogram pattern showed within normal limits in 46.43% of cases, 39.28%cases of left shift, right shift in 9.28% cases and bimodal in 5% of cases.

Conclusion: Peripheral smear examination is a sensitive and gold standard method in diagnosing red blood cell disorders. Red blood cell histogram patterns and the red cell indices obtained from the hematology automated analysers act as an adjunct to the visual examination of peripheral smears.

Keywords: Hematology analyser, peripheral smear, red cell indices, red cell histograms

Introduction

One of the most common nutritional problems affecting mothers and children in India is childhood anaemia. In terms of anaemia rates in earlier demographic and health surveys, private and state humanitarian measures to boost child health have made little progress [1].

The RBC histogram, in conjunction with RBC indices, Mean corpuscular volume, and RBC distribution width, is a crucial component of automated analysis and offers valuable insights in the diagnosis and treatment of red cell disorders [2].

Additionally, it provides crucial details that are not immediately obvious from the numerical data, such as in the case of megaloblastic anaemia with progressive iron shortage. The small population of microcytic hypochromic cells is recognised as double peak by the histogram [2]. A dual cell population is only indicated by a double peak histogram curve, which needs to be further connected with peripheral smear results.

Since MCV is a mean value and doesn't reflect the heterogeneity of the RBC population, further determination of MCV in a dimorphic population curve is useless [3]. In addition, knowledge of the abnormal curves of the RBC histogram and the flags is required to look for specific morphological features in peripheral smear for a correct diagnosis [3].

Despite the sophistication of modern instruments, initial calibration still requires the use of manual processes. This emphasises the value of manual technical skills despite the desire to leave everything up to automation.
There haven't been many research on the use of peripheral smear findings compared to RBC indices and histograms. In order to correlate these in order to improve the method of diagnosing anaemia, the current study is being conducted in the Department of Pathology, JNMC Belagavi.

Aims and Objectives

- Utility of red cell histograms in various types of anaemia in pediatric age group (1-18years)
- To correlate the peripheral smear impression with RBC histogram patterns

Materials and Methods

Source of data

Two hundred eighty patients with anemia in the age group 1-18 years as determined by decreased haemoglobin levels of less than the lower limit of the normal range for the particular age and sex at the diagnostic laboratory of Jawaharlal Nehru Medical College& KLEs Dr Prabhakar Kore Hospital and MRC were included in the present study.

- **Study design:** Cross sectional study.
- **Study period:** 1 year prospective (1st January 2021 to 31st December 2021)
- **Study population:** Patients in pediatric age group (1-18years) who are clinically diagnosed as anaemia in JNMC and KLE'S DR. PRABHAKAR KORE HOSPITAL and MRC from 1st January 2021 to 31st December 2021.

Inclusion criteria

All patients with haemoglobin levels of less than the lower limit of the range for the particular age and sex were included in the study.Patients of age group 1-18 years were included in the study.

Exclusion criteria

- Patients with haemoglobin levels greater than upper limit of the range for the particular age and sex
- Patients with leukaemia and platelet disorders.

Data collection: The present study will be conducted on cases with haemoglobin less than the lower limit of the normal range for the particular age and sex admitted at JNMC and KLES Dr. Prabhakar Kore Hospital Charitable Hospital and MRC, Belagavi.
After obtaining permission from the hospital authorities, the participants will be briefed about the study and informed consent will be taken from the prospective cases.

Data Analysis

The peripheral smear findings were evaluated for RBC morphology- RBC Anisocytosis and poikilocytosis were noted.

Anisocytosis

Anisocytosis is defined as variation in size greater than the normal. When the degree of variation is with in normal limits, cells are called as normocytic. When red cells are smaller than the nucleus of small lymphocytes they are called as microcytes. When they are larger than normal more than the nucleus of small lymphocytes, they are called macrocytes.

Grading the degree of anisocytosis

1. 1+ Anisocytosis-Most red cells in the average oil immersion field appear uniform in size but5-10 cells per field are easily distinguished from the predominant cell line.
2. 2+ Anisocytosis -10-15 cells per field are easily distinguished from the predominant cellline.
3. 3+ Anisocytosis-15-25 cells per field are easily distinguished from the predominant cellline.
4. 4+Anisocytosis-26-50 cells per field are easily distinguished from the predominant cell line.

Poikilocytosis: When the variation in shape is greater than normal, known as poikilocytosis.
If a particular abnormal shapes exceeds 5% of the cells percent, grading is done. Grading the degree of poikilocytosis

Results and Analysis

This prospective study was conducted from January 2021 to December 2021. The RBC Indices, histogram and peripheral smear of 280 patients having hemoglobin levels less than the lower limit of the normal range for the particular age and sex were analysed.

Age and gender wise distribution of anemia: Majority of female's i.e 61.97% (44 cases) were in the age group from 15 to 19 years. Females are more commonly affected than males in this study as depicted in the table below.

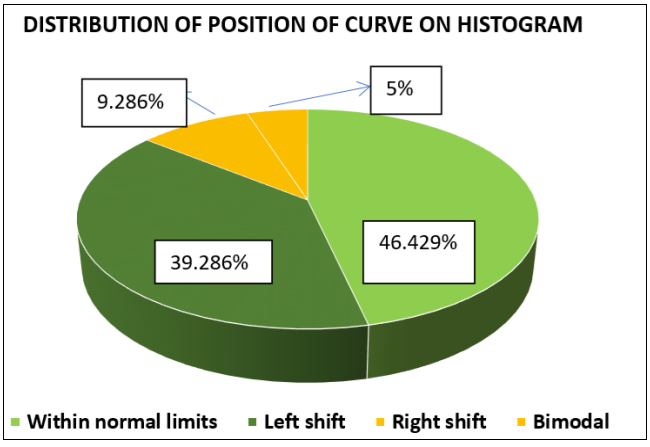
Table 1: Age and gender wise distribution.

Age	Female	%	Male	%	Total
0 - 4	3	37.50	5	62.50	8
5 - 9	19	46.34	22	53.66	41
10 - 14	94	58.75	66	41.25	160
15 - 19	44	61.97	27	38.03	71
Total	160	57.14	120	42.86	280

Histogram analysis

Position of curve on histogram analysis

Majority of histograms showed curves within normal limits 46.429% i.e 130 cases followed by shift to left in 39.286% (110 cases), 9.286% (26 cases) showed shift to right and only 5% (14 cases) showed bimodal pattern.



Graph 1: Position of curve on histogram analysis

Shape of curve on histogram analysis.
All 280 cases showed bell shaped curve (100%). Curve did not have broad base in majority 64.286% (180 cases), and broad base was present in only 35.714% (100 cases).

Table 2: Shape of curve on histogram analysis.

Bell Shape	Number	%
Present	280	100.000
Absent	0	0.000
Total	280	100.000

Table 3: Base of curve (Broadbase)

Broad base of curve	Number	%
Present	100	35.714
Absent	180	64.286
Total	280	100.000

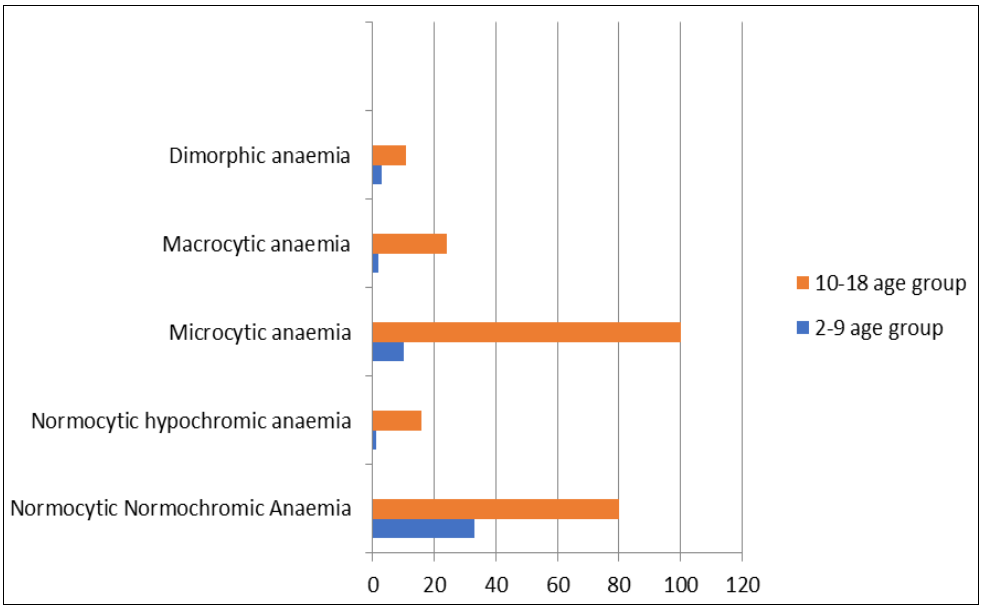
Impression based on histogram analysis/RBC indices
Further in our study, cases were analysed based on histogram and RBC indices. Majority 113 (40.357%) were normocytic normochromic anemia followed by Microcytic anaemiai.e110 (39.285%) cases. Macrocytic and dimorphic were9.286% and 5% respectively.

Table 4: Impression based on histogram analysis/RBC indices.

Impression on histogram analysis and rbc indices	Number	%
Normocytic Normochromic Anemia	113	40.357
Normocytic Hypochromic Anaemia	17	6.071
Microcytic Hypochromic Anemia	110	39.285
Macrocytic Anemia	26	9.286
Dimorphic Anemia	14	5.000
Total	280	100.000

Table 5: Impression on histogram and RBC indices according to age group

	Age		
Impression On Histogram Analysis And Rbc Indices	**2 - 9**	**10 - 18**	**Total**
Normocytic Normochromic Anemia	33	80	113
Normocytic Hypochromic Anaemia	1	16	17
Microcytic Hypochromic Anemia	10	100	110
Macrocytic Anemia	2	24	26
Dimorphic Anemia	3	11	14
Total	49	231	280



Graph 2: Impression on histogram and RBC indices according to age group

On analysis of histogram and RBC indices, we found that microcytic hypochromic anaemia is predominantly seen in the age group of 10-18 years followed by normocytic normochromic anaemia. In 2-9 years of age, normocytic normochromic anaemia is more common in our study.
For the above table, the value of p has been calculated using chi square test. P value is 0.0015 which is statiscally significant in this study.

Peripheral Smear Examination

Anisocytosis on peripheral smear examination

Anisocytosis score 1 was seen in majority of cases 144 cases (51.42%) followed by 103 cases (36.78%) which showed score 2. Score 3 was seen in least no of cases 22(9.286%). Remaining 11(3.9%) cases showed score 4.

Table 6: Anisocytosis on peripheral smearexamination

Anisocytosis Score	Number	%
1	144	51.42
2	103	36.78
3	22	7.8
4	11	3.9
Total	280	100.000

Poikilocytosis on peripheral smear examination

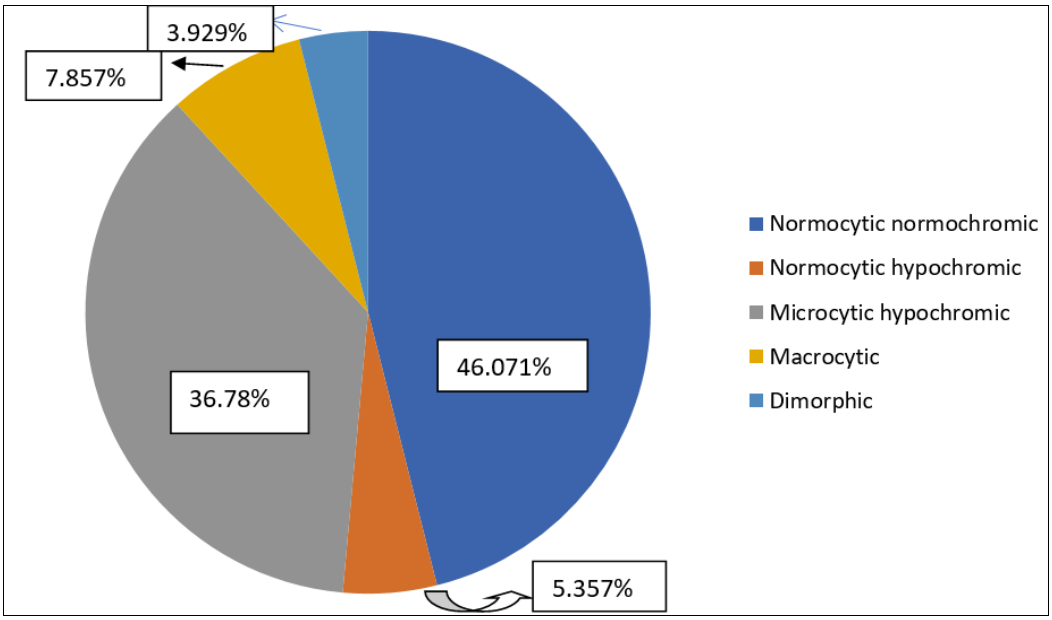
Majority of cases showed mild poikilocytosis 151 cases (53.929%). Moderate poikilocytosis was seen in 117(41.78%) of cases. Only few 11 cases (3.92%) showed severe poikilocytosis.

Table 7: Poikilocytosis on peripheral smear examination.

Poikilocytosis	Number	%
Mild Poikilocytosis	151	53.929
Moderate Poikilocytosis	117	41.78
Severe Poikilocytosis	11	3.92
Total	280	100.000

Impression based on peripheral smear examination

Majority 129 cases (40.357%) were normocytic normochromic anemia. Microcytic, macrocytic and dimorphic were 36.78%, 9.286% and 5% respectively.



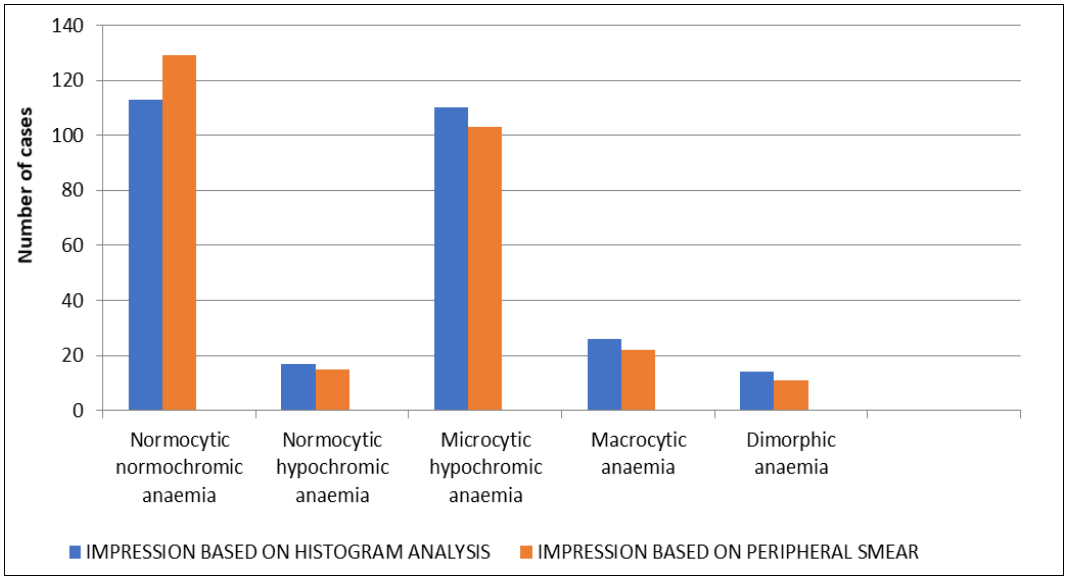
Graph 3: Morphological type of anemia based on peripheral smear

Table 8: Morphological type of anemia according to age group

Age			
Morphological Type of Anaemia Based On Peripheral Smear	**2 - 9**	**10 - 18**	**Total**
Normocytic Normochromic Anemia	29	100	129
Normocytic Hypochromic Anaemia	1	14	15
Microcytic Hypochromic Anemia	10	93	103
Macrocytic Anemia	3	19	22
Dimorphic Anemia	4	7	11
Total	47	233	280

On peripheral smear analysis we found that normocytic normochromic anaemia is predominantly seen in the age group 10-18 years followed by microcytic hypochromic anaemia. In 2-9 years, normocytic normochromic anaemia is more common in our study.

For the above correlation, p value has been calculated using chi square test which showed statistically significant result with p value less than 0.0001.



Graph 4: Correlation between impression on histogram analysis and on peripheral smear

Further impression made on the morphological type of anaemia by Peripheral smear vs RBC histogram and indices were statistically analysed. The correlation between the morphological types of anaemia based on Peripheral smear vs RBC histogram and indices was analyzed using Cramer's V(0.523) where the value is 0.9302 with $p<0.0001$, which was statistically significant. Kappa statistics for agreement between the two methods=0.9078(moderate agreements), p<0.0001. Both these suggest there is high degree of agreement between the two procedures of assessment.

Discussion

Age

In our study the population taken into consideration is 2-18 years mean age being 12.14. It can be compared to similar study done by Prakash A *et al*., Bhushan D Kamble *et al*. and Rosemerry Ferreira *et al*.

Table 9: Comparision of patients in different age group

Age Group	Prakash A *et al*. [4]	Bhushan D Kamble *et al*. [5]	Present study
0-4	40.7%	61.7%	2.86%
5-9	30.7%		14.64%
10-14	8.6%	46.3%	57.14%
15-18	20%		25.36%

In our study we have found out that majority of the cases having anaemia are in the age group 10-14 years whereas in the study by Prakash A *et al*. showed the predominance of anaemia is in the age group 0-4 years. The reason for the discordance might be the small sample size of the former study which included only 150 participants. Our present study is also in concordance with a study by Bhushan DK amble *et al*. which showed that majority of the anaemia is below the age group of 15 years. Another study by Rosemerry Ferreira *et al*. showed most of the anaemic cases are less than one year. But limitation of the study is that it considered population only under 5 years. So this study was discordant with our study.

Sex distribution

The total numbers of cases of anemia in our study was 280. Out of these majority were females 160 (57.14%) and 120 (42.86%) were males. This is comparable to the previous studies done as shown in table below.

Table 10: Comparison of sex distribution

Sex	Anisetti & Komuravelli (2021) [7]	Ingale SV *et al*. (2017) [8]	Present Study
Female	52.81%	61.5%	57.14%
Male	47.19%	38.5%	42.86%

In our study it was seen that majority were female patients which are in the age group 10-18 years. These results were in concordance with the studies conducted by Anisetti & Komuravelli and Ingale SV *et al*. The prevalence of anaemia was higher among females than males, as would be expected because of blood loss during these reproductive age.

Peripheral Smear Examination: Present study showed out of 280 cases, majority were normocytic normochromic patients. This was compared to other studies as shown in table below**.**

Table 11: Comparison of peripheral smear findings with similar studies.

Peripheral smear findings	Anisetti & Komurav-elli (2021) [7]	Prakash A *et al*. [4]	Kumar *et al*. (2013) [9]	Saba *et al*. (2014) [1]	Present study (2021)
Normocytic normochromic anemia	32.20%	-	31.7%	22%	46.42%
Microcytic hypochromic anemia	40.82%	98.7%	33.3%	49%	36.07%
Macrocytic anemia	3.74%	1.3%	21.7%	4%	7.857%
Dimorphic anemia	23.22%	-	13.3%	24%	3.93%

In the present study, the maximum cases were of normocytic normochromic anaemia. This is in discordant with the other studies due to lesser sample size in the former studies.

The other morphoplogical types of anaemia found in the study was in corcordance with the referred studies in the table.

The above comparison shows that the second most common morphological type of anemia was Microcytic hypochromic anaemia. Iron deficiency is the most common cause of microcytic hypochromic blood picture. The reason for this includes chronic iron deficiency, malabsorption, parasitic infestations etc. Furthermore in adolescent girls, menstruation may be the major contributing factor for the microcytic hypochromic anaemia. Prevalence of anemia in all groups is higher in India as compared to other developing countries.

Iron nutrition is a matter of continuing concern in both developing and developed countries of the world. A reduction in the amount of dietary iron and its availability has made iron a borderline nutrient in modern society[12].

Correlation of diagnosis of anemia based on peripheral smear vs Histogram and RBC indices analysis.

The current study was double-blind study. First, peripheral blood smear analysis was performed without consulting the histogram or indices. Later, without conducting an independent peripheral smear examination, the diagnosis is determined using the RBC histogram and RBC indices.

Table 12: Showing diagnosis of anemia made on Peripheral Smear vs Histogram and indices

Type of Anemia	On peripheral smear analysis	On histogram and RBC indices analysis
Normocytic normochromic anemia	129	113
Normocytic hypochromic anemia	15	17
Microcytic hypochromic anemia	103	110
Macrocytic anemia	22	26
Dimorphic anemia	11	14

Correlation of Peripheral smear vs RBC histogram and Indices in Microcytic Hypochromic Anemia

Only 36.429% of cases had microcytic anaemia on peripheral smear examination, despite the fact that histogram and RBC indices analysis revealed that 39.28% of cases shifted to the left with MCV range from 46.4fl-79.9fl supporting this. This can be explained because automated analyzers are known to make mistakes when giant platelets are present, when hemolytic diseases cause fragmented RBCs, when platelet clumps are present, and when microerythrocytes are present because the machine misinterprets them as microcytes and produces falsely high values. Peripheral smear rules out these errors.

Correlation studies in Normocytic Normochromic Anemia: On peripheral smear examination 129 out of 280 cases were normocytic normochromic anemia. On histogram analysis 130 cases showed position within normal limits and MCV ranged from 87.4-100.7fl. So diagnosis based on RBC indices and histograms were comparable to diagnosis with peripheral smear examination. It is comparable to study done by Kumar A *et al*. (2013) [9]. The causes for this might be early iron deficiency or any subclinical infection. The other causes are nutritional deficiencies, acute blood loss, hemolysis or any renal diseases.

Correlation studies in Macrocytic Anemia

Macrocytic anemia was seen in 22 out of 280 cases on peripheral smear examination. But histogram and RBC indices showed 78 cases as macrocyticanemia. Although the MCV measured by an automated blood cell counter is rarely wrong, conditions including hyperglycemia, marked leukocytes, and cold agglutinins might cause the MCV to be incorrectly elevated. Furthermore, partial instrument obstruction and many hours of room temperature storage of the blood samples might potentially cause a misleading rise of the MCV value. This study is consistent with a study conducted by Florence *et al*. in that a small number of individuals identified as macrocytic on histogram and RBC indices analysis were identified as dimorphic on peripheral smear examination [14].

Correlation studies in Dimorphic Anemia

Out of 280 cases, only 11 cases were diagnosed as dimorphic anaemia in peripheral smear.

Dimorphic anaemia showed a variety of histogram patterns, from simple curve to complex curve. The majority of the curves were tilted to the left and had wide bases. The broad base may be explained by the presence of numerous populations of cells of differing sizes (i.e.normocytic, microcytic and macrocytic). Peripheral smears of the majority of patients showed a combination of microcytes and macrocytes, with microcytes predominating. Our study agreed with Constantino *et al*. findings. 's from 2010 [2].

The dimorphic blood picture will appear as a dual population of microcytic and normocytic or normocytic and macrocytic red cells or as a mixture of small, normal, and large cells of different sizes and forms with or without normal red blood cell indices, so it is crucial to examine the peripheral smear to look at all the cell populations. Practically speaking, morphological findings should be coupled with the graphical and numerical data for better interpretation of results since the dimorphic blood picture is often associated with aberrant red cell populations.

In 2013 Naveen *et al*. presented similar findings for the dual

population of RBCs. He asserted that the dual RBC population can be recognised using the histogram's most striking representation [15].

Conclusion

The automated haematology analyzer's red cell histograms and RBC indices offer important insights into haematological diseases like anaemia. RBC histograms have received very little attention in research, which have placed more emphasis on WBC histogram and leukemic blast populations. In identifying different morphological kinds of anaemia, our study found a substantial association between RBC histograms and RBC indices versus peripheral smear diagnosis.

Bimodal peaks in the histograms suggested the presence of two RBC populations. However, a peripheral smear was required to assess the same. The RBC indicators didn't increase help with the identification of these situations, as MCV showed that the size of the current RBC populations.

Thus, it was determined that peripheral smear test, together with clinical history, is a significant diagnostic tool when treating patients in the era of molecular analysis and automation. The visual evaluation of peripheral smears is supplemented by the use of red cell histograms and numerical metrics such MCV, MCH, MCHC, and RDW.

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