Journal of Hematology and Blood Disorders
Volume 12 | Issue 1

Research Article Open Access

Comparative Study of Peripheral Smear with RBC Indices and RBC Histograms in Cases of Anemia in Adults

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Citation: Sreya Sujil, Lakshmi Priya (2026) Comparative Study of Peripheral Smear with RBC Indices and RBC Histograms in Cases of Anemia in Adults. J Hematol Blood Disord 12(1): 101

Received Date: February 15, 2026 **Accepted Date:** March 06, 2026 **Published Date:** March 09, 2026

Abstract

Background: Anaemia is a common haematological disorder characterized by a reduced red blood cell (RBC) count, haemoglobin level, or haematocrit, leading to impaired oxygen transport. Proper classification of anaemia is crucial for effective diagnosis and treatment. Traditionally, anaemia is classified using RBC indices mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), and red cell distribution width (RDW) which provide essential quantitative information about RBC morphology and size distribution.

Objective: To evaluate the diagnostic accuracy of peripheral smear examination in detecting different types of anaemia. To assess the role of RBC histograms in identifying anaemia subtypes and their correlation with peripheral smear and RBC indices.

Materials and Methods: "Comparative study of peripheral smear with RBC indices and RBC histograms in cases of anaemia in adults" is a cross sectional study. Material of the study will be obtained from the blood samples received in the Haematology lab, Department of Pathology, SBMCH, Chrompet, Chennai. Relevant clinical details will be taken from request forms

Result: The RBC histogram is an exceptionally powerful screening tool that provides an immediate visual representation of the cell population, often flagging dimorphic or mixed populations that single numerical indices might average out.

Conclusion: This study confirms that automated hematology analyzers provide highly accurate data that strongly correlates with manual peripheral blood smear findings. MCV and RBC Histograms emerged as the most critical automated parameters, showing highly significant statistical associations with manual morphological classification.

Keywords: Anemia, Peripheral Blood Smear, RBC Indices, RBC Histogram, Mean Corpuscular Volume (MCV),

Automated Hematology Analyzer

Introduction

Anemia is a medical condition characterized by a lower-than-normal number of red blood cells or a decreased concentration of hemoglobin in the blood. Red blood cells contain hemoglobin, a protein responsible for carrying oxygen from the lungs to all parts of the body. When the number of red blood cells or hemoglobin levels drop, the blood's capacity to transport oxygen to tissues is reduced, leading to various symptoms associated with inadequate oxygen supply. Anemia can range from mild to severe and may develop either temporarily or over a longer period. It is a common blood disorder across the globe and can affect anyone but is particularly prevalent among young children, women of reproductive age, and pregnant women. This condition is significant because it not only causes symptoms like fatigue, weakness, dizziness, and shortness of breath, but it may also signal the presence of other underlying health issues that require attention. In severe cases, anemia can be life-threatening. As a clinical sign rather than a disease itself, anemia underscores the importance of evaluating an individual's overall health and nutritional status to identify and address the root causes effectively. The condition's impact on quality of life and public health is substantial, leading to widespread efforts to prevent, diagnose, and manage anemia through nutritional, medical, and public health interventions worldwide

Material S and Methods

"Comparative study of peripheral smear with RBC indices and RBC histograms in cases of anaemia in adults" is a cross sectional study. Material of the study will be obtained from the blood samples received in the Haematology lab, Department of Pathology, SBMCH, Chrompet, Chennai. Relevant clinical details will be taken from request forms

Inclusion criteria: Adult patients (ages 18–60 years) diagnosed with anaemia based on clinical and laboratory criteria.

Exclusion criteria: Patients with acute infections, haematological disorders other than anaemia, or recent blood transfusions.

Data Collection Tools

Patient history

Blood samples (EDTA Vacutainer)

Sampling Procedure

Anticoagulant: Collect 2–3 mL of venous blood into an EDTA (Ethylene diamine Tetra acetic Acid) tube to prevent clotting.

Sample Timing: Use fresh samples ideally within 2 hours of collection to prevent morphological distortion of red cells.

Sources of Blood: Venous blood from the antecubital vein is commonly used; in infants, heel or finger prick samples may also be used.

Preparation of Peripheral Blood Smear

Slide Preparation: Place a small drop of blood near one end of a clean glass slide. Using a spreader slide at a 30–45° angle, spread the blood smoothly to create a thin film with a feathered edge . Allow the smear to air dry completely; avoid blowing or using heat.

Fixing & Staining

Stain the dried smear using Leishman or Wright–Giemsa stain for about 5–10 minutes wash gently with buffered water (pH 6.8) and air dry again.

Microscopic Examination

Examine under an oil immersion microscope to assess:RBC morphology: size, colour, and shape (normocytic, microcytic, macrocytic, hypochromic, etc.)Presence of abnormal cells: target cells, spherocytes, schistocytes, sickle cells, etc. .Findings are correlated with automated RBC indices such as: Mean Corpuscular Volume (MCV): average cell size. Mean Corpuscular Hemoglobin (MCH): hemoglobin content per cell. Mean Corpuscular Hemoglobin Concentration (MCHC): average hemoglobin concentration within cells.Red Cell Distribution Width (RDW): degree of variation in cell size .

Correlation with RBC Indices

Automated analyzers provide RBC histograms and indices that classify anemia (microcytic, normocytic, macrocytic).

Peripheral smear evaluation complements these data by identifying morphological abnormalities that automated machines may miss useful in dimorphic anemia, hemolytic anemia, and abnormal cell morphologies.

Result And Discussion

The collected data were entered in the Microsoft Excel 2016 and analysed with IBM SPSS Statistics for Windows, Version 29.0. (Armonk, NY: IBM Corp). To describe about the data descriptive statistics frequency analysis, percentage analysis were used for categorical variables and the mean & S.D were used for continuous variables. To find the significant difference between the multivariate analysis the Kruskal Walli's test followed by the Mann-Whitney U test was used was used. To find the significance in qualitative categorical data Chi-Square test was used. In all the above statistical tools the probability value .05 is considered as significant level.

Age distribution where 18 – 20 yrs is 8.3%, 21 – 30 yrs is 12.5%, 31 – 40 yrs is 6.3%, 41 – 50 yrs is 20.8%, 51 – 60 yrs is 10.4%, 61 – 70 yrs is 16.7%, 71 – 80 yrs is 25.0%. The minimum age recorded is 18, the maximum age is 80, and the mean age ± standard deviation is 52.25±19.70 years.

Comparison of Hb_g/dL between Peripheral smear by Kruskal-Wallis test where KW ⊠ 2 – value= 1.637, p = 0.651>0.05, with mean±SD of Normocytic is (9.51±1.77), & Median, IQR is (9.60,2.25), mean±SD of Microcytic is (9.57±1.85), & Median, IQR is (9.75,2.90), mean±SD of Macrocytic is (9.02±1.90), & Median, IQR is (8.30,2.70) and mean±SD of Dimorphic is (10.30±0.66), & Median, IQR is (10.40,-9.60) which shows no statistically significant difference at $p > 0.05$ level.

Comparison of MCV_fL between Peripheral smear by Kruskal-Wallis test followed by the Mann-Whitney U test where KW ⊠ 2 – value= 64.737, p = 0.0005>0.05, with mean±SD of Normocytic is (88.24±5.09), & Median, IQR is (87.75,7.25), mean±SD of Microcytic is (76.39±3.56), & Median, IQR is (76.85,3.28), mean±SD of Macrocytic is (105.56±3.06), & Median, IQR is (106.50,4.45) and mean±SD of Dimorphic is (93.97±1.27), & Median, IQR is (94.60,-92.50) which shows a highly statistically significant difference at $p < 0.01$ level, Followed by the Mann-Whitney U test shows highly statistically significant difference at $p < 0.01$ level for Normocytic with Microcytic, Normocytic with Macrocytic, Microcytic with Macrocytic and Microcytic with Dimorphic and statistically significant difference at $p < 0.05$ level for Normocytic with Dimorphic and Macrocytic with Dimorphic.

Comparison of PCV_% between Peripheral smear by Kruskal-Wallis test where KW ⊠ 2 – value= 4.998, p = 0.172>0.05, with mean±SD of Normocytic is (28.72±6.85), & Median, IQR is (28.95,8.93), mean±SD of Microcytic is (29.32±6.10), & Median,

IQR is (29.55,10.03), mean±SD of Macrocytic is (22.90±4.25), & Median, IQR is (20.60,7.85) and mean±SD of Dimorphic is (30.50±2.54), & Median, IQR is (29.40,-28.70) which shows no statistically significant difference at $p > 0.05$ level.

Comparison of MCH_pg between Peripheral smear by Kruskal-Wallis test where KW ⊠ 2 – value= 1.946, $p = 0.584 > 0.05$, with mean±SD of Normocytic is (28.07±4.01), & Median, IQR is (28.35,6.18), mean±SD of Microcytic is (27.88±5.03), & Median, IQR is (27.65,4.63), mean±SD of Macrocytic is (30.44±3.54), & Median, IQR is (32.70,6.45) and mean±SD of Dimorphic is (26.77±6.25), & Median, IQR is (25.90,-21.00) which shows no statistically significant difference at $p > 0.05$ level.

Comparison of MCHC_g/dL between Peripheral smear by Kruskal-Wallis test where KW ⊠ 2 – value= 1.609, $p = 0.657 > 0.05$, with mean±SD of Normocytic is (33.25±1.75), & Median, IQR is (33.40,2.60), mean±SD of Microcytic is (33.21±1.62), & Median, IQR is (33.00,2.45), mean±SD of Macrocytic is (32.20±2.35), & Median, IQR is (31.90,4.35) and mean±SD of Dimorphic is (32.87±0.75), & Median, IQR is (32.90,-32.10) which shows no statistically significant difference at $p > 0.05$ level.

Comparison of RDW between Peripheral smear by Kruskal-Wallis test where KW ⊠ 2 – value= 4.585, $p = 0.205 > 0.05$, with mean±SD of Normocytic is (18.24±2.95), & Median, IQR is (18.00,4.00), mean±SD of Microcytic is (18.79±6.57), & Median, IQR is (17.00,13.63), mean±SD of Macrocytic is (16.60±5.49), & Median, IQR is (15.50,8.25) and mean±SD of Dimorphic is (22.67±3.06), & Median, IQR is (22.00,-20.00) which shows no statistically significant difference at $p > 0.05$ level.

Comparison of Peripheral smear between RBC Histogram by Pearson's Chi-Square test where $⊠^2=288$, $p=0.0005 < 0.01$, which shows a highly statistically significant association between Peripheral smear and RBC Histogram.

Table 1: Age Distribution.

Age distribution		
	Frequency	Percent
18 - 20 yrs	8	8.3
21 - 30 yrs	12	12.5
31 - 40 yrs	6	6.3
41 - 50 yrs	20	20.8
51 - 60 yrs	10	10.4
61 - 70 yrs	16	16.7
71 - 80 yrs	24	25
Total	96	100

Table 2: Gender Distribution.

Gender distribution		
	Frequency	Percent
Female	48	50
Male	48	50
Total	96	100

Table 3: Comparison of Hb g/dL between Peripheral smear by Kruskal-Wallis Test.

Variable	Peripheral Smear	N	Mean	S. D	Median	IQR	KW ⊠ 2 - value	p-value
Hb_g/dL	Normocytic	62	9.51	1.77	9.6	2.25	1.637	0.651 #
	Microcytic	26	9.57	1.85	9.75	2.9		
	Macrocytic	5	9.02	1.9	8.3	2.7		
	Dimorphic	3	10.3	0.66	10.4	-9.6		
# No Statistical Significance at p > 0.05 level								

Table 4: Comparison of MCV_fL between Peripheral smear by Kruskal-Wallis test followed by the Mann-Whitney U Test.

Variable	Peripheral smear	N	Mean	S.D	Median	IQR	KW ⊠ 2 - value	p-value
MCV_fL	Normocytic	62	88.24	5.09	87.75	7.25	64.737	0.0005 **
	Microcytic	26	76.39	3.56	76.85	3.28		
	Macrocytic	5	105.56	3.06	106.5	4.45		
	Dimorphic	3	93.97	1.27	94.6	-92.5		
** Highly Statistical Significance at p < 0.01 level								

Table 5: Comparison of PCV_% between Peripheral smear by Kruskal-Wallis test.

Variable	Peripheral smear	N	Mean	S.D	Median	IQR	KW ⊠ 2 - value	p-value
PCV_%	Normocytic	62	28.72	6.85	28.95	8.93	4.998	0.172 #
	Microcytic	26	29.32	6.1	29.55	10.03		
	Macrocytic	5	22.9	4.25	20.6	7.85		
	Dimorphic	3	30.5	2.54	29.4	-28.7		
# No Statistical Significance at p > 0.05 level								

Table 6: Comparison of MCH_pg between Peripheral smear by Kruskal-Wallis test.

Variable	Peripheral smear	N	Mean	S.D	Median	IQR	KW ⊠ 2 - value	p-value
MCH_pg	Normocytic	62	28.07	4.01	28.35	6.18	1.946	0.584 #
	Microcytic	26	27.88	5.03	27.65	4.63		
	Macrocytic	5	30.44	3.54	32.7	6.45		
	Dimorphic	3	26.77	6.25	25.9	-21		
# No Statistical Significance at p > 0.05 level								

Table 7: Comparison of MCHC_g/dL between Peripheral smear by Kruskal-Wallis test.

Variable	Peripheral smear	N	Mean	S.D	Median	IQR	KW ⊠ 2 - value	p-value
MCHC_g/dL	Normocytic	62	33.25	1.75	33.4	2.6	1.609	0.657 #
	Microcytic	26	33.21	1.62	33	2.45		
	Macrocytic	5	32.2	2.35	31.9	4.35		
	Dimorphic	3	32.87	0.75	32.9	-32.1		
# No Statistical Significance at p > 0.05 level								

Table 8: Comparison of RDW between Peripheral smear by Kruskal-Wallis Test.

Variable	Peripheral smear	N	Mean	S.D	Median	IQR	KW 2 - value	p-value
RDW	Normocytic	62	18.24	2.95	18	4	4.585	0.205 #
	Microcytic	26	18.79	6.57	17	13.63		
	Macrocytic	5	16.6	5.49	15.5	8.25		
	Dimorphic	3	22.67	3.06	22	-20		
# No Statistical Significance at p > 0.05 level								

Table 9: Comparison of Peripheral smear between RBC Histogram by Pearson's Chi-Square test.

			RBC Histogram				Total	2 - value	p-value
			Broad base	Left shift	Normal curve	Right shift			
Peripheral smear	Normocytic	Count	0	0	62	0	62	288	0.0005 **
		%	0	0	1	0	1		
	Microcytic	Count	0	26	0	0	26		
		%	0	1	0	0	1		
	Macrocytic	Count	0	0	0	5	5		
		%	0	0	0	1	1		
	Dimorphic	Count	3	0	0	0	3		
		%	1	0	0	0	1		
Total		Count	3	26	62	5	96		
		%	0.031	0.271	0.646	0.052	1		
** Highly Statistical Significance at p < 0.01 level									

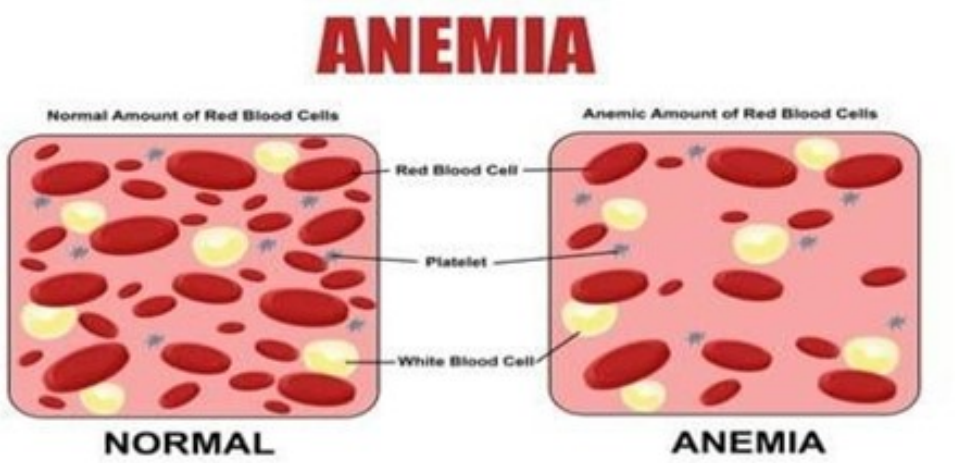

Figure 1: Anemia

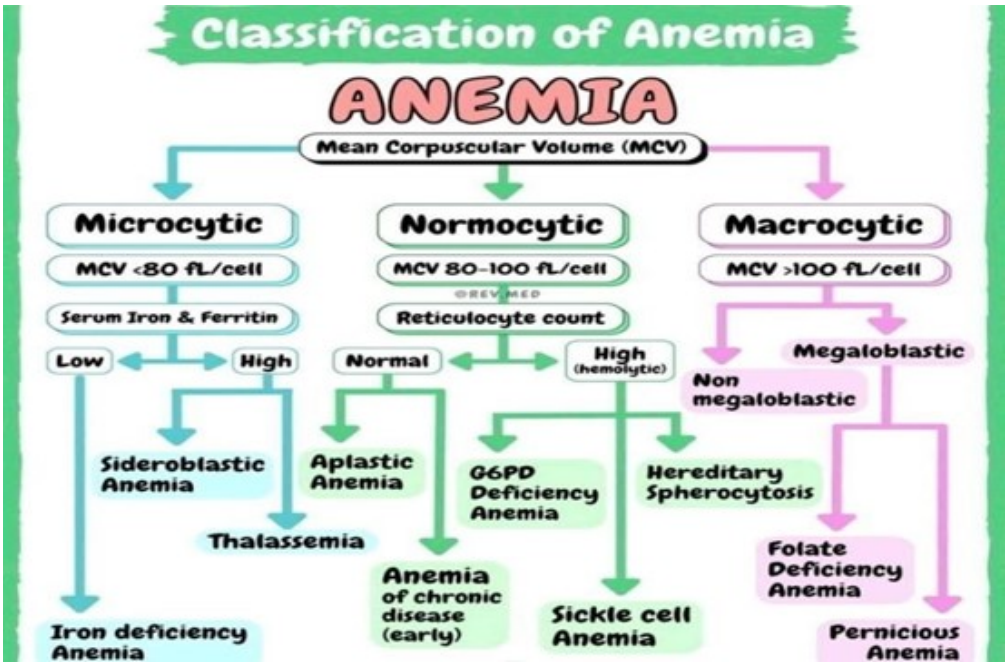

Figure 2: Classification of Anemia.

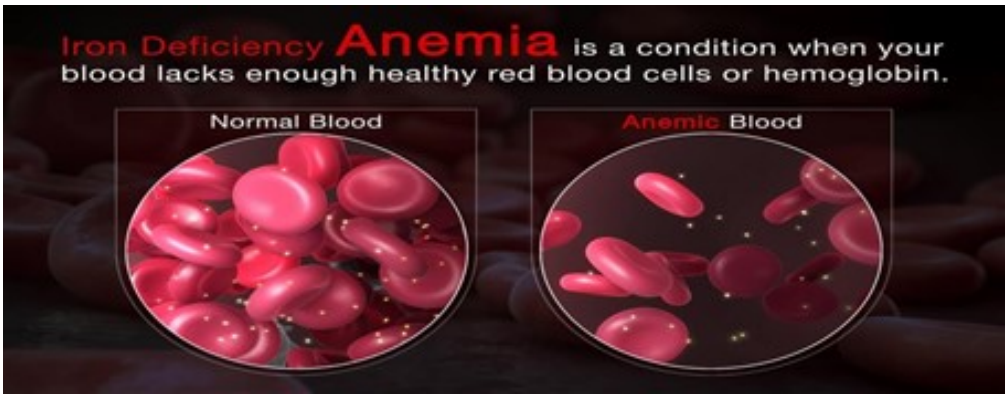

Figure 3: Iron Deficiency Anemia.

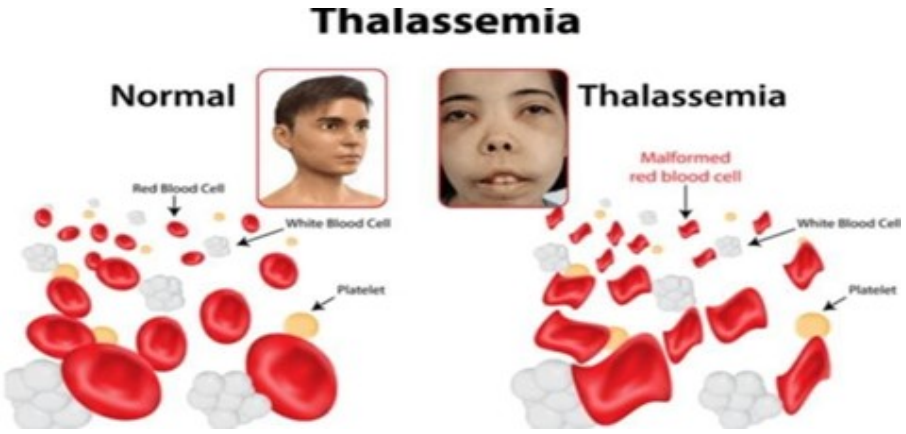

Figure 4: Thalassemia.

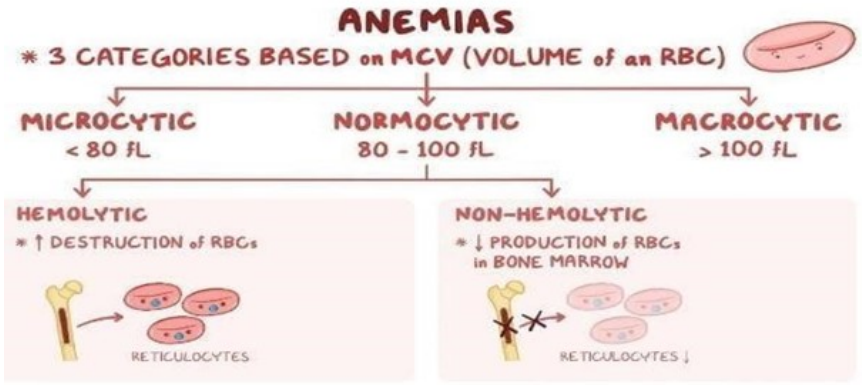

Figure 5: Categories Based On MCV

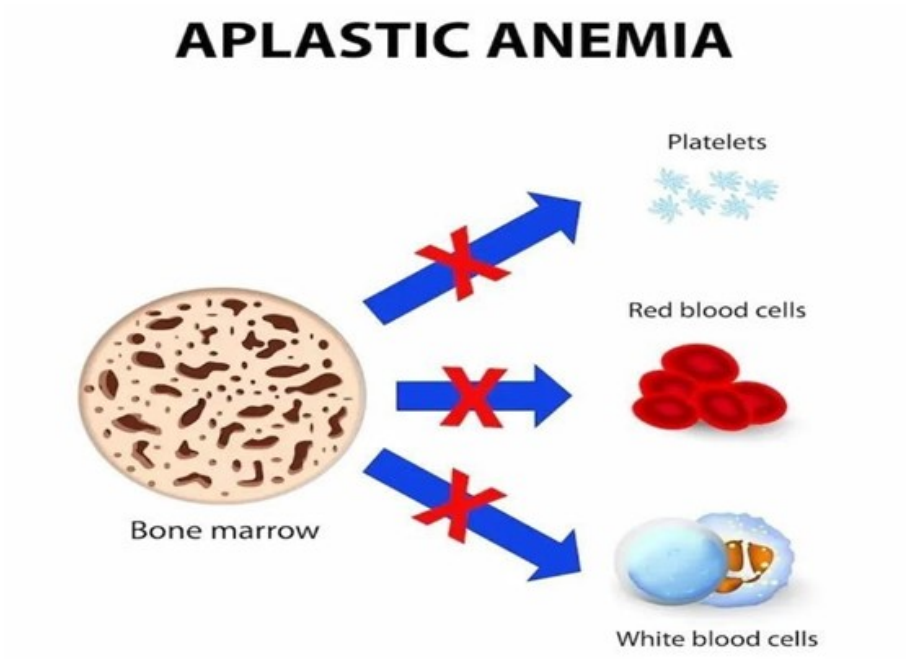

Figure 6: Aplastic Anemia

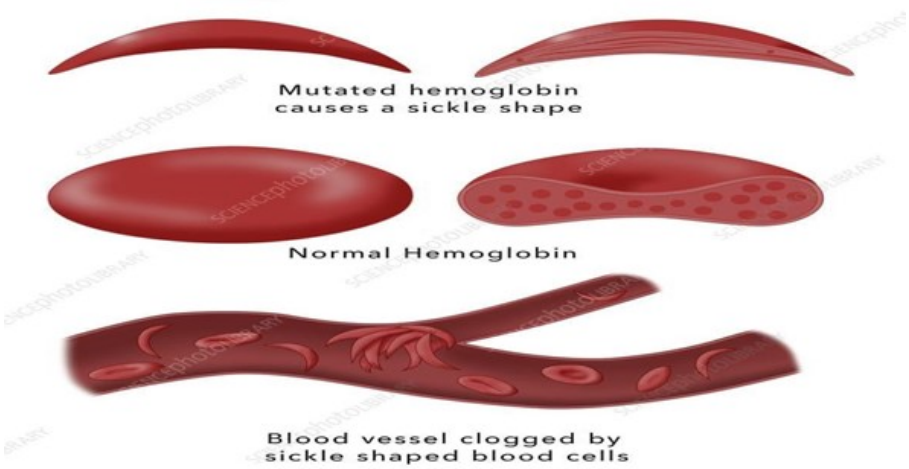

Figure7: Sickle cell Anemia.

Discussion

The primary aim of this study was to evaluate the correlation between manual peripheral smear examination and automated parameters (RBC indices and histograms) in the diagnosis of anemia in adults. The study population of 95 adults showed an equal gender distribution (50% male, 50% female) and a broad age range, with a significant concentration of cases (25%) in the elderly population (71–80 years), reflecting the high prevalence of anemia in older adults.

Correlation with RBC Indices

The study found that Mean Corpuscular Volume (MCV) is the most reliable automated index for classifying anemia, showing a highly statistically significant difference ($p = 0.0005$) across morphological types. Normocytic cases had a mean MCV of 88.24 fL, microcytic cases 76.39 fL, and macrocytic cases 105.56 fL, which aligns with standard clinical classifications.

Interestingly, parameters such as Hemoglobin (Hb), Packed Cell Volume (PCV), MCH, MCHC, and RDW did not show statistically significant differences ($p > 0.05$) between the morphological categories in this specific study group. This suggests that while these indices are vital for identifying the presence and severity of anemia, they may be less effective than MCV and histograms at differentiating between specific morphological types in a mixed adult population.

The Diagnostic Value of RBC Histograms one of the most significant findings of this research is the 100% correlation between RBC histograms and peripheral smear findings ($p = 0.0005$).

Normal Curve histograms perfectly matched Normocytic smears.

Left Shift histograms identified all Microcytic cases.

Right Shift histograms identified all Macrocytic cases.

Broad Base histograms were exclusive to Dimorphic anemia.

This highly significant association indicates that the RBC histogram is an exceptionally powerful screening tool that provides an immediate visual representation of the cell population, often flagging dimorphic or mixed populations that single numerical indices might average out.

Conclusion

This study confirms that automated hematology analyzers provide highly accurate data that strongly correlates with manual peripheral blood smear findings. MCV and RBC Histograms emerged as the most critical automated parameters, showing highly significant statistical associations with manual morphological classification. The 100% agreement between histogram patterns (shifts and base width) and smear morphology proves that histograms are an essential first-line diagnostic tool. However, the study also reinforces that while automation offers speed and high-volume screening, the peripheral smear remains indispensable. It is vital for confirming complex cases, such as dimorphic anemia, and for identifying specific morphological abnormalities like poikilocytosis or inclusions that automated systems cannot yet fully characterize. In summary, a combined approach utilizing both the precision of automated RBC histograms/indices and the descriptive detail of manual smears ensures the most comprehensive and accurate diagnosis of anemia in adult patients.

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