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ORIGINAL RESEARCH

Correlation of Peripheral Smear with RBC Indices and RBC Histogram in the Diagnosis of Anemia

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Abstract
Background: Anemia, a widespread global health issue, poses significant challenges in both developing and developed countries. Accurate diagnosis and classification of anemia are crucial for effective management and treatment. This study explores the correlation between automated hematology analyzers and peripheral blood film (PBF) examination in classifying anemia types. **Methods:** A cross-sectional study was conducted on 200 consecutive anemic patients. EDTA-anticoagulated blood samples were analyzed using the HORIBA Pentra DX Nexus automated hematology analyzer and examined manually via PBF. Key parameters including mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), and red cell distribution width (RDW) were assessed. Concordance between automated results and PBF findings was analyzed using the kappa statistic. **Results:** Normocytic normochromic anemia with normal RDW was the most common, observed in 39% of both RBC indices and PBF findings. Normocytic hypochromic anemia was identified in 5% of the indices and 6% of PBF cases. Microcytic hypochromic anemia was consistent across both methods, seen in 30% of cases. Macrocytic anemia was noted in 12% of both indices and PBF results. Dimorphic anemia was exclusively identified by PBF in 13% of cases, with no corresponding findings from the analyser. The study found an 86% agreement rate between automated and manual methods, with a kappa value of 0.721, indicating substantial correlation. PBF identified 13 cases of dimorphic anemia not detected by the analyzer. These findings highlight the complementary value of PBF in diagnosing complex anemia types. **Conclusion:** Both automated analyzers and PBF are essential for comprehensive anemia diagnosis. While automated methods provide efficient routine classification, PBF remains vital for detecting complex anemia types. The integration of both approaches ensures accurate and thorough evaluation of anemia.
Keywords: Anemia, automated hematology analyzer, peripheral blood film (PBF), red blood cell indices, morphological classification, diagnostic correlation, dimorphic anemia, Kappa statistics

Introduction

Anaemia, a condition characterized by a deficiency in red blood cells (RBCs) or haemoglobin, impairs the body's ability to deliver adequate oxygen to tissues and organs. Anaemia represents a significant global public health challenge, impacting both developing and developed nations, with profound implications for individual health, as well as societal and economic development.[1] According to the World Health Organization (WHO), the threshold for normal haemoglobin concentration is defined as 12.0 g/dL for women and 13.0 g/dL for men at sea level.[2]Anaemia itself is not a standalone diagnosis but rather an indicator of an underlying condition. Consequently, the evaluation of anaemia focuses on identifying the root causes of decreased red blood cell counts and associated morphological alterations.[3,4] Accurate diagnosis and classification of anaemia are crucial for effective treatment and management. Automated haematologyanalysers have revolutionized the initial assessment of anaemia by providing rapid and comprehensive blood counts, including haemoglobin levels, haematocrit, mean corpuscular volume (MCV), and red cell distribution width (RDW).However, these automated devices, while essential, have limitations in assessing the detailed morphology of RBCs.[5] This is where the peripheral blood smear examination remains indispensable. The blood smear allows for a manual review of RBC morphology, which is vital for diagnosing specific types of anaemia. For instance, the smear can reveal anisocytosis (variation in RBC size), poikilocytosis (variation in RBC shape), and the presence of abnormal inclusions—features that are crucial for identifying conditions like iron deficiency anaemia, thalassaemia, or sickle cell disease.[6]Additionally, the peripheral blood smear provides valuable information on other haematological abnormalities, such as abnormal white blood cells or platelet morphology,

which can be indicative of secondary causes of anaemia or other underlying disorders.[7] Thus, while automated haematologyanalysers offer efficiency and standardization, peripheral blood smear examination enhances diagnostic accuracy by offering a detailed view of RBC characteristics, ensuring a more comprehensive understanding of anaemia and related conditions.The present study aims to evaluate the utility of peripheral blood smear examination alongside automated haematology analyzers in diagnosing anaemia. By correlating anaemia types identified through RBC indices from automated analyzers with peripheral blood smear findings, the study seeks to enhance diagnostic accuracy.

Methods

This cross-sectional study was conducted at the Department of Pathology, Govt. Medical College, Jammu, to assess the utility of peripheral blood smear examination alongside automated haematology analyzers in diagnosing anaemia. A total of 200 consecutive samples from anemic patients were collected for this study. The study included patients with haemoglobin levels below 11 g/dL. Exclusion criteria were applied to samples that were clotted or hemolysed, and to patients who declined to provide consent for participation. Blood samples, each 2 ml in volume, were drawn into EDTA vials. These samples were then processed for two distinct analyses: one portion was used with a hematology analyzer to obtain red blood cell indices, while the other portion was used for peripheral blood smear examination.Each patient's detailed clinical history was meticulously recorded, and comprehensive data was compiled.The samples were analyzed using the HORIBA Pentra DX Nexus, a seven-part automated haematology analyzer, and peripheral blood smears were prepared concurrently. The haematological investigations included a Complete Blood Count (CBC) performed on the automated analyzer, which provided measurements of hemoglobin, total erythrocyte count, and red cell indices such as mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and red cell distribution width (RDW). Peripheral blood smears, stained with Leishman's stain, were examined in detail using low power (10x), high power (40x), and oil immersion lenses (100x). The study was approved by the Institute of Ethical Clearance, and informed consent was obtained from all participants.

Results

In this section, the results of the study will be described:

Table 1: Age distribution of study patients

Age (Years)	Number	Percentage
21-30 Years	41	20.5
31-40 Years	78	39.0
41-50 Years	37	18.5
51-60 Years	30	15.0
> 60 Years	14	7.0
Total	200	100
Mean±SD (Range)=38.3±5.78 (22-78 Years)		

Out of 200 anemic patients included in the present study, the majority were aging between 31 and 40 years old, comprising 39% of the cohort. The next largest group was aged 21-30 years, accounting for 20.5% of the sample. Patients aged 41-50 years made up 18.5%, while those in the 51-60 years bracket constituted 15%. The smallest group was patients older than 60 years, at 7%. The mean age of the studied population was 38.3 years with a standard deviation of 5.78 years, spanning a range from 22 to 78 years. Females represented 56.5% of the sample, while males comprised 43.5%. The gender ratio was 1.29 females for every male. The most common clinical presentation among the patients was pallor, observed in 48.5% of cases. A significant proportion of patients exhibited mild anemia, with 46% having this degree of severity. The average hemoglobin level in the cohort was 9.4 g/dL, with a standard deviation of 2.34 g/dL.

Table 2: RBC size among study patients as measured by MCV on analyser

RBC Size	No.	%age
Normocytes	115	57.5
Microcytes	60	30.0
Macrocytes	25	12.5
Total	200	100

We measure RBC size in the blood samples by MCV on analyzer wherein,normocytes were identified in 57.5% of the patients, while 30% were classified as microcytes. Macrocytes were observed in 12.5% of the study population. This distribution highlights the varying RBC sizes among the anemic patients.

Table 3: Chromia among study patients as measured by MCHC on analyser		
Chromia	**No.**	**%age**
Normochromia	127	63.5
Hypochromia	73	36.5
Total	200	100

The table 3 outlines the chromia, or color intensity, of RBCs as determined by mean corpuscular hemoglobin concentration (MCHC). Normochromia was present in 63.5% of patients, indicating a normal color intensity of RBCs. Hypochromia was observed in 36.5% of the patients, reflecting reduced color intensity due to lower hemoglobin content per RBC.

Table 4: Anisocytosis among patients as measured by the RDW on Analyser		
Anisocytosis	**No.**	**%age**
Present	86	43.0
Absent	114	57.0
Total	200	100

Anisocytosis was present in 43% of patients, indicating variability in RBC size. Conversely, 57% of the patients did not exhibit anisocytosis, suggesting uniform RBC sizes.

Table 5: Morphological type of anemia on the basis of analyser generated RBC Indices and on PBF examination				
Morphological type of Anemia	**RBC Indices with RDW (Analyser)**			**PBF**
	Normal RDW	**Raised RDW**	**Total**	
Normocytic normochromic	39	11	50	39
Normocytic hypochromic	5	3	8	6
Microcytic hypochromic	6	24	30	30
Macrocytic anemia	8	4	12	12
Dimorphic anemia	-	-	-	13
Total	58	42	100	100

The table 5 compares the morphological types of anemia identified through RBC indices from the analyzer and peripheral blood film (PBF) examination. Normocytic normochromic anemia with normal RDW was the most common, observed in 39% of both RBC indices and PBF findings. Normocytic hypochromic anemia was identified in 5% of the indices and 6% of PBF cases. Microcytic hypochromic anemia was consistent across both methods, seen in 30% of cases. Macrocytic anemia was noted in 12% of both indices and PBF results. Dimorphic anemia was exclusively identified by PBF in 13% of cases, with no corresponding findings from the analyser.

Table 6: Correlation between the type of anemia by analyser generated RBC parameters and PBF examination	
Type of anemia on analyser (%age of patients)	**Type of anemia on PBF (%age of patients)**
Normocytic normochromic anemia with normal RDW (39%)	Normocytic normochromic anemia (39%)
Normocytic hypochromic anemia with normal RDW (5%)	Normocytic hypochromic anemia (6%)
Microcytic hypochromic anemia (30%)	Microcytic hypochromic anemia (30%)
Macrocytic anemia (12%)	Macrocytic anemia (12%)
Normocytic anemia with raised RDW (14%)	Dimorphic anemia (13%)
Kappa (95% CI) = 0.721 (0.525-0.916)	

The assessment of correlation between the type of anemia by analyser generated RBC parameters and PBF examination revealed a strong correlation, with 86% (172) of cases showing agreement between the two methods. The kappa statistic for this correlation was 0.721, with a 95% confidence interval of 0.525 to 0.916, indicating a substantial level of agreement between the analyzer and PBF findings.

Discussion

The age distribution of the study population highlighted a notable prevalence of anemia in individuals aged 31-40 years, accounting for 39% of the sample. This age group, while relatively young, is critical as it represents a stage of life associated with significant socio-economic responsibilities and reproductive health considerations. The mean age of 38.3 years underscores that anemia is particularly impactful during mid-adulthood, a period often characterized by increased health challenges and demands. These findings align with existing literature, such as the study by Hafiz F et al., which reported that 58% of their anemic patients were in the 21-40 year age range, reinforcing our observations.[7-10]Gender distribution in our study revealed a higher prevalence of anemia in females (56.5%) compared to males (43.5%). This trend is consistent with known higher rates of anemia in women, often attributed to menstrual losses, nutritional deficiencies, and reproductive health issues. Our findings are corroborated by Swami S et al., who reported that 73.86% of their cohort were females, and by Hafiz et al., who found a predominance of females (55%) with a female-to-male ratio of 1.22:1. This supports the observation that women are more frequently affected by anemia.[7,11]

Clinically, pallor was the most common presentation, observed in 48.5% of the patients. This finding is consistent with previous research, including the studies by Chandurkar M et al. and Hafiz F et al., the latter of which identified pallor as the most common clinical presentation in 45% of cases.[7,12] The high prevalence of pallor underscores its value as a key clinical indicator of anemia. Additionally, a significant proportion of patients (46%) exhibited mild anemia, with an average hemoglobin level of 9.4 g/dL and a standard deviation of 2.34 g/dL. These results highlight the need for effective screening and management strategies tailored to the prevalent forms and severity of anemia in the studied population.The analysis of red blood cell (RBC) parameters, including red cell distribution width (RDW), mean corpuscular hemoglobin (MCH), and mean corpuscular volume (MCV), was instrumental in diagnosing and classifying anemia in our study. Among the RBC parameters, normocytes were the most prevalent, identified in 57.5% of cases, followed by microcytes at 30% and macrocytes at 12.5%. These results indicate a predominance of normocytic and microcytic anemia types, which are essential for differentiating the underlying causes of anemia.These findings are in line with those reported by Swami L et al. and Hafiz F et al.[7,11] Specifically, Hafiz F et al. identified normocytes (MCV 80-100 fL) in the majority (59%) of their patients, with a mean MCV of 85.84 fL. They also reported that microcytic anemia (MCV <80 fL) and macrocytic anemia (MCV >100 fL) constituted 31% and 10% of cases, respectively.[7] This aligns well with our observations, underscoring the significance of RBC parameter measurements in accurately diagnosing and categorizing anemia types.

The assessment of chromia, as measured by Mean Corpuscular Hemoglobin Concentration (MCHC), revealed that normochromia was present in 63.5% of patients, while hypochromia was observed in 36.5%. The high prevalence of hypochromia indicates a notable proportion of patients may have iron deficiency or other conditions that result in decreased hemoglobin content per red blood cell (RBC). This observation is significant as hypochromia is commonly associated with various forms of anemia, particularly those related to iron deficiency. These findings align with the results from Singla S et al. and Hafiz F et al.[7,13] Specifically, Hafiz F reported that normochromia was found in 61% of patients, and hypochromia in 39%, based on MCHC measurements.[13] This consistency with our study underscores the utility of MCHC in diagnosing and differentiating anemia, particularly in identifying potential iron deficiency or related disorders. Anisocytosis, as measured by the analyzer, was observed in 43% of patients, indicating variability in red blood cell (RBC) size. This finding reflects a notable degree of heterogeneity in RBC dimensions among a significant portion of the study population. Conversely, 57% of patients did not exhibit anisocytosis, suggesting a more uniform RBC size distribution. The mean Red Cell Distribution Width (RDW) in our study was 18.25%, which aligns with findings from Sandhya V et al, who reported an RDW range of 12.4% to 39.2%, with a mean of 19.3%.[14] Similarly, Hafiz F reported an RDW range of 11.6% to 33%, with a mean value of 17.15%.[7] These results are consistent with the detection of anisocytosis in 41% of cases reported by Hafiz F, underscoring the relevance of RDW as a key parameter in assessing variability in RBC size and diagnosing anemia.

The classification of anemia types using both automated RBC indices and peripheral blood film (PBF) examination revealed varying degrees of concordance between the two methods. Notably, the concordance rate for normocytic normochromic anemia was 78%, with 39 of 50 cases consistently identified by both the analyzer and PBF. This result aligns with findings from Kaur H et al., who reported normocytic normochromic anemia as the predominant type in 56% of their cases.[15] Similarly, Hafiz F et al. observed that 40% of patients exhibited normocytic normochromic anemia with normal RDW, on both analyzer and PBF, corroborating our results.[7]For normocytic hypochromic anemia, a 75% concordance rate was observed, with 6 out of 8 cases identified by both methods. This is indicative of a significant level of agreement but also highlights some discrepancies in RDW interpretation. Conversely, microcytic hypochromic anemia showed a 100% concordance rate, as all 30 cases identified by the analyzer were confirmed by PBF. This high level of agreement underscores the reliability of both methods in identifying microcytic hypochromic anemia. Similarly, macrocytic anemia demonstrated a 100% concordance rate,

with all 12 cases identified by the analyzer being confirmed by PBF. This suggests that both methods are equally effective in diagnosing macrocytic anemia. However, dimorphic anemia was exclusively detected by PBF, with no corresponding cases identified by the analyzer's RDW assessment. This discrepancy suggests that the automated analyzer may not be sensitive to certain types of anemia, emphasizing the need for comprehensive diagnostic approaches.The findings of this study revealed a robust correlation between analyzer-generated RBC indices and peripheral blood film (PBF) examination, evidenced by an impressive agreement rate of 86%. The kappa statistic of 0.721, with a 95% confidence interval ranging from 0.525 to 0.916, indicates a substantial alignment between these two diagnostic modalities. This statistic reinforces the reliability of automated analyzers in the routine classification of anemia while highlighting the indispensable role of PBF examination in identifying more intricate anemia types. The observed agreement is consistent with prior research. For instance, Hafiz F et al. reported a kappa value of 0.545, signifying moderate agreement between automated and manual methods, with a statistically significant p-value of 0.0001, supporting our study's findings.[7] Similarly, Sandhya V et al noted comparable results, emphasizing the congruence of automated and manual classifications.[14] Our study also underscores the additional diagnostic value of PBF examination. Specifically, PBF identified 13 cases of dimorphic anemia that were not detected by the automated analyzer. This discrepancy highlights the limitations of automated methods, which may overlook complex anemia types characterized by multiple distinct red blood cell populations. The importance of PBF in these scenarios aligns with the findings of Hafiz F et al., who noted that PBF provided additional information in 16% of anemia cases, and Singal S et al., who reported a similar figure of 11.4%.[7,13] These results are further supported by Froom P et al. and Novis DS et al., who found additional diagnostic value in 13.9% and 6.4% of anemia cases, respectively.[16,17] Overall, while automated analyzers offer efficiency and consistency in anemia diagnosis, this study demonstrates that PBF remains an essential tool, particularly for detecting and diagnosing more complex anemia conditions that automated methods may miss.

Conclusion

The present study effectively assessed anemia through a combination of automated hematology analyzers and peripheral blood film (PBF) examinations, offering a nuanced understanding of its prevalence, types, and diagnostic accuracy. The data revealed that anemia predominantly affects individuals in mid-adulthood, with a notable higher prevalence among females, consistent with established patterns related to menstrual and nutritional factors. Clinical findings emphasize that pallor is a common presentation, and the majority of patients exhibit mild to moderate anemia. The study's analysis of red blood cell morphology indicated a range of anemia types, with significant findings related to normocytic, microcytic, and macrocytic classifications. Notably, PBF examination identified cases of dimorphic anemia that automated methods missed, underscoring the value of manual assessment in detecting complex anemia types. Overall, the strong correlation between automated indices and PBF examination, coupled with substantial agreement metrics, highlights the efficacy of both diagnostic approaches. This integrated methodology ensures comprehensive anemia diagnosis and improves patient care through enhanced diagnostic accuracy.

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