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COMPARATIVE STUDY OF MORPHOLOGICAL SPECTRUM OF RED BLOOD CELL BY AUTOMATED ANALYZER GENERATED RED BLOOD CELL PARAMETER WITH PERIPHERAL BLOOD SMEAR IN ANAEMIA

Pathology

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ABSTRACT

Background: Red blood cell (RBC) indices obtained from automated analyzer give an idea about morphological changes in anaemia. Along with the peripheral smear, they can be used to interpret the cause of anaemia. The aim of the present study was to find out the utility of automated haematology analyzer generated Red cell parameters in comparison to peripheral blood smear examination for study of morphological spectrum of anaemia. **Material And Methods:** A cross-sectional study was conducted over one year in the Department of Pathology at Maharaja Agrasen Medical College, Agroha, Haryana. Blood samples collected from 250 anaemic patients in the Hematology laboratory, were typed for anaemia, first by parameters obtained by haematology analyzer (RBC indices with red cell distribution width) and then by peripheral blood smear findings. The association between the diagnosis made by peripheral smear versus RBC indices and histogram were analysed to find out the significance of the mean difference in various parameters. **Results:** In our study females accounted for a higher proportion, comprising 58.4%, while males represented 41.6% with male: female ratio of 1:1.4. The most common peripheral smear finding in females was microcytic hypochromic anaemia (49.3%), followed by normocytic normochromic anaemia (28.8%). In males, microcytic hypochromic anaemia was also the most frequent (38.5%), with normocytic normochromic anaemia following closely (30.8%). Automated analyser findings showed high concordance with PBS in microcytic hypochromic anaemia (95.6%) and haemolytic anaemia (100%), while accuracy was moderate for dimorphic (88.9%), normocytic normochromic (76.3%), and macrocytic anaemia (70.4%). **Conclusion:** Histogram and RBC indices give valuable information, but peripheral smear examination remains the important diagnostic tool in haematological disorders.

KEYWORDS

Anaemia, Automated analyzer, Peripheral blood smear, Histogram, Red blood cell parameter.

INTRODUCTION

Anaemia is a widespread public health problem associated with an increased risk of morbidity and mortality despite of the fact that this can be mainly avoidable & painlessly treatable.[1] WHO defines anaemia as a reduction in red blood cell count or haemoglobin level below the normal percentage for that age.[2] According to National Family Health Survey (NFHS-5) 2019-2021; the prevalence of anaemia in Haryana was 70.4% in children aged 6–59 months, 60.4 % in females aged 15–49 years, and 18.9% in males aged 15–49 years.[3] In order to characterize the type of anaemia and formulate a differential diagnosis, the workup should include haematological parameters, i.e. haemoglobin, hematocrit, red blood cell indices like the red blood cell count, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), red cell distribution width (RDW), RBC histogram along with peripheral smear examination to evaluate cell morphology.[1] Despite this wealth of information, there are still morphological abnormalities that are critical in the differential diagnosis of anaemia and that can be determined only from a blood smear. The present study aims to find out the utility of automated haematology analyzer generated Red cell parameters in comparison to peripheral blood smear examination in diagnosis of different types of Anaemia.[4]

AIM AND OBJECTIVE:

To find out the utility of automated haematology analyzer generated Red cell parameters in comparison to peripheral blood smear examination for study of morphological spectrum of anaemia.

Inclusion Criteria

1. Patients diagnosed with anaemia based on WHO Haemoglobin Cut-off values from blood samples received for CBC and PBF examination.
2. Patients of all age groups and genders were included.

Exclusion Criteria

1. Clotted samples were not considered.
2. Samples insufficient for analysis in the analyzer were excluded.
3. Patients with leukemoid reaction and leukaemia were excluded.
4. Non-anaemic patients were not included in the study.

MATERIAL & METHOD:

This cross-sectional study was conducted over one year in the Department of Pathology at Maharaja Agrasen Medical College, Agroha, Haryana between November 2023 to November 2024. The study involved 250 confirmed cases of anaemia identified through Complete Blood Count (CBC) and Peripheral Blood Smear (PBS) analysis. The Study was carried out after getting ethical clearance from the Institutional ethics committee.

Patients were selected using systematic random sampling, and 2 ml of peripheral venous blood was collected in EDTA vacutainers for analysis. The blood samples were examined using the SYSMEX XN-550 automated haematology analyzer to measure red cell indices such as haemoglobin, RBC count, haematocrit, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), and red cell distribution width (RDW). Peripheral blood smears were prepared using Leishman's stain and analyzed microscopically. Reticulocyte counts were assessed using New Methylene Blue staining. The position and the shape of the RBC histogram were recorded. The automated red cell parameter findings were compared with PBS results for anaemia classification.

OBSERVATIONS & RESULTS

Among the 250 study participants, the majority were in the 21–30 years age bracket (26.4%), followed by the 31–40 years group

(21.2%). Participants aged ≤10 years accounted for the smallest proportion (4.8%). Females accounted for a higher proportion, comprising 58.4%, while males represented 41.6% with male: female ratio of 1:1.4.

Table I. Distribution Of Study Participants According To Their Peripheral Smear Findings (n=250)

Peripheral smear	Female	Male	p-value
Microcytic hypochromic	72 (49.3)	40 (38.5)	0.054
Normocytic normochromic	42 (28.8)	32 (30.8)	
Macrocytic anaemia	6 (4.1)	13 (12.5)	
Dimorphic anaemia	19 (13)	10 (9.6)	
Haemolytic anaemia	7 (4.8)	9 (8.7)	
Total	146 (100)	104 (100)	

The most common peripheral smear finding in females was microcytic hypochromic anaemia (49.3%), followed by normocytic normochromic anaemia (28.8%). In males, microcytic hypochromic anaemia was also the most frequent (38.5%), with normocytic normochromic anaemia following closely (30.8%). Other findings included macrocytic, dimorphic, and haemolytic anaemia, with no statistically significant differences between sexes (p = 0.054).

Table II. Distribution Of Study Participants According To Their Autoanalyzer Parameter (n=250)

Autoanalyzer parameter	Female	Male	p-value
Microcytic hypochromic with raised RDW	69 (47.3)	38 (36.5)	0.142
Microcytic hypochromic with normal RDW	3 (2.1)	3 (2.9)	
Normocytic normochromic with raised RDW	35 (24)	26 (25)	
Normocytic normochromic with normal RDW	21 (14.4)	15 (14.4)	
Macrocytic anaemia	10 (6.8)	17 (16.3)	
Haemolytic anaemia	3 (2.1)	1 (1)	
Dimorphic anaemia	5 (3.4)	4 (3.8)	
Total	146 (100)	104 (100)	

Among females, the most common anaemia type detected was microcytic hypochromic anaemia with raised red cell distribution width (RDW), observed in 47.3% of cases. This was followed by normocytic normochromic anaemia with raised RDW in 24% and normocytic normochromic anaemia with normal RDW in 14.4%. In contrast, among males, microcytic hypochromic anaemia with raised RDW was present in 36.5% of cases, followed by normocytic normochromic anaemia with raised RDW (25%) and normocytic normochromic anaemia with normal RDW (14.4%). Macrocytic anaemia was significantly more common in males (16.3%) than in females (6.8%). Dimorphic anaemia was noted in 3.4% of females and 3.8% of males, while haemolytic anaemia was identified in 2.1% of females and 1% of males. The statistical analysis revealed no significant differences between the genders across the different anaemia categories (p = 0.142).

Table III. Distribution Of Study Participants According To Histogram Pattern (n=250)

Histogram pattern	Frequency	Percentage
Left shift	62	24.8
Right shift	14	5.6
Normal curve	80	32
Bimodal	9	3.6
Broad curve	85	34
Total	250	100

Histogram analysis revealed that a broad curve was the most frequent abnormality, occurring in 34% of participants. Left shift was observed in 24.8%, while a normal curve was present in 32%. A right shift and bimodal pattern were found in 5.6% and 3.6%, respectively.

Table IV. Accuracy Of Autoanalyzer In Diagnosing Different Types Of Anaemia As Compared To Peripheral Smear (n=250)

Autoanalyzer	PBS	Frequency	Percentage	p-value
Dimorphic anaemia (n=9)	Dimorphic	8	88.9	0.028*
	Haemolytic	1	11.1	
Haemolytic anaemia (n=4)	Haemolytic	4	100	-
Macrocytic anaemia (n=27)	Macrocytic	19	70.4	<0.001*
	Dimorphic	3	11.1	
	Haemolytic	5	18.5	
Microcytic hypochromic (n=113)	Microcytic hypochromic	108	95.6	<0.001*
	Haemolytic	1	0.9	
	Dimorphic	4	3.5	
Normocytic normochromic (n=97)	Normocytic normochromic	74	76.3	<0.001*
	Microcytic hypochromic	4	4.1	
	Haemolytic	5	5.2	
	Dimorphic	14	14.4	

In the 9 patients with dimorphic anaemia as per autoanalyzer, PBS confirmed dimorphic anaemia in 8 of them, yielding an accuracy of 88.9%. On the other hand all 4 of the patients identified to be haemolytic anaemia as per autoanalyzer were correctly identified as such by PBS (100% accuracy). For 27 patients with macrocytic anaemia as per autoanalyzer, the accuracy of diagnosis was 70.4% as per PBS. While 108 (95.6%) of the 113 patients diagnosed with microcytic hypochromic anaemia as per autoanalyzer were confirmed to be as such by PBS. For those identified to have normocytic normochromic anaemia as per the autoanalyzer results, 74 (76.3) were confirmed to having had such as per PBS, while the other diagnoses were microcytic hypochromic and dimorphic anaemia respectively.

DISCUSSION

The present study analyzed the demographic distribution, severity, and diagnostic categorization of anaemia using automated haematology analyzers in comparison with peripheral blood smear (PBS) examination. The majority of anaemia cases were observed in the 21–30 years age group (26.4%), followed by 31–40 years (21.2%), while the lowest prevalence occurred in children ≤10 years (4.8%). These findings are consistent with Singh et al[8], who reported higher prevalence among young adults, especially females. The greater burden in this age group may be explained by increased physiological demands, nutritional inadequacies, and reproductive factors in women. In the present study, females (58.4%) were more affected than males (41.6%), similar to observations by Kouli et al[1] and Revathi et al[9].

Anaemia severity distribution showed moderate anaemia as most common (43.2% females; 37.5% males), while severe anaemia was more frequent in females (26%) compared to males (17.3%). Although the difference between genders was not statistically significant (p = 0.051), the findings reflect the disproportionate physiological burden on women of childbearing age, as also noted by Revathi et al[9].

Diagnostic evaluation using automated analyzers revealed variability in performance across anaemia types. Reticulocytosis was most marked in haemolytic anaemia (3 ± 0.6%), indicating increased RBC turnover. Haemoglobin levels were lowest in haemolytic anaemia (7.1 ± 2.1 g/dl) and highest in normocytic normochromic anaemia (10.9 ± 1.5 g/dl), findings are consistent with Hafiz et al[7], who reported that automated analyzers cannot identify morphological features such as schistocytes and spherocytes. Macrocytic anaemia showed the highest mean MCV (110.8 ± 10.5 fl), consistent with Singh et al[8]. The autoanalyzer demonstrated excellent diagnostic accuracy for microcytic hypochromic anaemia (sensitivity 95.6%, specificity 97.1%) and good accuracy for normocytic normochromic anaemia (sensitivity 76.3%, specificity 100%). However, performance was lower for macrocytic (sensitivity 70.4%) and dimorphic anaemia (sensitivity 88.9%, specificity 91.3%), with significant discordance noted in mixed populations, as also reported by Singhal et al[10]. In haemolytic anaemia, although sensitivity was high (100%), but the positive predictive value was low (25%), indicating limitations in detecting morphological features such as spherocytes and schistocytes. These results support the necessity of PBS examination in suspected haemolytic conditions, as highlighted by Hafiz et al[7].

Histogram analysis was found useful in preliminary anaemia classification. Broad histogram curves, reflecting anisocytosis and dimorphic patterns, were most common (34%), followed by left-shifted curves (24.8%) correlating with microcytic anaemia. Right-

shifted (5.6%) and bimodal (3.6%) patterns were less frequent but clinically significant, with bimodal curves strongly suggestive of dimorphic anaemia which often requires additional PBS examination for confirmation. These findings align with Kerdany et al[11] and Garg et al[2], who emphasized the correlation of histogram broadening and left-shifts with iron deficiency. However, histograms lack the sensitivity to detect subtle morphological abnormalities, underscoring their complementary rather than definitive role in anaemia classification, as also noted by Singhal et al[10].

PBS examination confirmed microcytic hypochromic anaemia as the most common subtype (49.3% females, 38.5% males), consistent with Phukan et al[12] and Garg et al[2]. This high prevalence is attributable to widespread iron deficiency from inadequate intake, chronic blood loss, and reproductive demands. PBS provided additional diagnostic value by identifying target cells, pencil cells, and stippling suggestive of thalassemia, which cannot be discerned from automated parameters alone. Normocytic normochromic anaemia was the second most frequent type (28.8% females, 30.8% males), comparable to Singh et al[8]. This subtype often reflects chronic disease or marrow suppression, with PBS enabling identification of rouleaux formation or teardrop cells in relevant conditions. Macrocytic anaemia was less common, more frequent in males, and associated with nutritional deficiencies or alcoholism, requiring PBS to distinguish megaloblastic from non-megaloblastic causes. Dimorphic anaemia showed significant under-detection by the autoanalyzer compared to PBS (13% females and 9.6% males on smear vs. 3.4% females and 3.8% males on analyzer), consistent with Singhal et al[10] (11.4%) reflecting the inability of automated systems to differentiate mixed cell populations. Haemolytic anaemia was also more reliably identified on PBS (4.8% females, 8.7% males) whereas the automated analyzer detected (2.1% females and 1% males), findings align with Hafiz et al[7], reinforcing the role of peripheral smear in diagnosing red cell fragmentation and polychromasia.

CONCLUSION

The study concluded that automated haematology analyzers provided rapid and accurate detection of microcytic hypochromic and normocytic normochromic anaemia but showed limitations in dimorphic, macrocytic, and haemolytic subtypes. Histogram analysis offered useful preliminary insights, particularly for iron deficiency and mixed patterns, but PBS remained indispensable for morphological confirmation. Together, automated analyzers and PBS form a complementary diagnostic approach, ensuring greater accuracy and aiding effective clinical management of anaemia.

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