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Comparative analysis of peripheral smear and automated counter findings in anemia among pregnant women: A retrospective study

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Abstract:
Pregnancy anemia poses serious maternal-fetal risks in developing nations, yet automated hematology analyzers versus peripheral smear accuracy for morphological subtyping remains insufficiently compared in prenatal care. Therefore, it is of interest to analyze the 120 antenatal cases, correlating hemoglobin levels and red cell indices from automated counters with peripheral smear morphological findings for anemia classification accuracy. Automated analyzers showed high correlation with smear results for basic parameters but showed discrepancies in morphological subtyping requiring microscopic confirmation. Peripheral smear examination proved essential for precise anemia subtype identification, complementing automated data for optimal clinical decision-making. Thus, data shows the combined automated-peripheral smear protocols are essential for accurate prenatal anemia diagnosis and targeted maternal-fetal management.

Keywords: Anemia in pregnancy, peripheral smear, automated hematology analyzer, red cell indices, morphological classification, diagnostic accuracy

Background:
Worldwide, anemia during pregnancy is a major cause of maternal and perinatal death and morbidity. It is a common hematological condition. Nearly 40% of pregnant women worldwide have anemia during pregnancy, with the greatest frequency seen in low- and middle-income nations, according to the World Health Organization (WHO) **[1]**. Hemoglobin values below 11 g/dL are considered anemia in pregnancy. Nutritional inadequacies, socio-economic issues and poor healthcare access contribute to the disproportionately high incidence of anemia in India **[2]**. Iron deficiency is the most common cause of anemia during pregnancy, but other factors such as folate and vitamin B12 deficiencies, chronic infections, hemoglobinopathies and chronic infections may also contribute **[3]**. Hematological parameters are already difficult to evaluate during pregnancy because of the many physiological changes that occur, such as hemodilution from an increase in plasma volume **[4]**. In order to effectively treat anemia and minimize complications, it is crucial to accurately diagnose and classify the condition. For a long time, a peripheral blood smear was the gold standard for diagnosing anemia. It gives specific morphological details on RBCs, such as their size, shape, color and if they have any abnormalities or inclusions **[5]**. Different morphological forms of anemia may aid in diagnosis and treatment planning, including microcytic hypochromic, normocytic normochromic, macrocytic and dimorphic anemia **[6]**. Automated hematology analyzers have found extensive usage in clinical practice as a result of technological improvements in laboratories. In a nutshell, these analyzers provide quantitative data including RDW, MCV, MCH and hemoglobin concentration in a short amount of time with high degree of precision and repeatability **[7]**. These indices help with the initial diagnosis by allowing microcytic, normocytic and macrocytic kinds of anemia to be classified **[8]**. Automated analyzers have several limits, yet they nonetheless offer many benefits. Even in situations of early nutritional deficits or dimorphic anemia, they can miss morphological defects, mixed anemia, or aberrant cell populations **[9]**. Misclassification of anemia types may also occur due to specific artifacts and technological constraints. Therefore, it is possible to get inaccurate diagnoses when using just automated parameters. By visually validating the shape of red blood cells, peripheral smear inspection supplements automated results. Anisocytosis, poikilocytosis, hypochromia, macrocytosis and aberrant cells such nucleated RBCs and target cells may be easily detected using it **[10]**. In addition, it is crucial for the detection of hemoglobinopathies and hemoparasites, which might be missed by automated analyzers **[11]**. In order to improve diagnosis accuracy, recent investigations have highlighted the significance of linking automated hematological parameters with the results of peripheral smears. For pregnant women, when correct categorization is critical for proper treatment, a combination approach improves subtype identification of anemia and aids in avoiding misdiagnosis **[12]**. Discrepancies were most often seen in mixed or borderline situations, but studies have shown variable degrees of concordance between the two approaches **[13]**. Therefore, it is of interest to emphasize the significance of an integrated diagnostic strategy by analyzing the level of concordance and diagnostic accuracy, therefore revealing the advantages and disadvantages of both methodologies.

Materials and Methodology:
Study design:
Retrospective observational study

Study setting:
Tertiary care center Gandhi medical college and Hamidia Hospital, Bhopal over a period from March 2024 to March 2025 was the site of the research.

Study population:
Pregnant women diagnosed with anemia who had hematological tests throughout the research period.

Sample size:
In all, 120 instances of anemia in pregnant women were considered for the research. Complete laboratory data, including

results from automated hematology analyzers and peripheral smear testing, allowed for the retrospective selection of these patients.

Inclusion criteria:

[1] Pregnant women with hemoglobin levels <11 g/dL.
[2] Cases with complete hematological data, including automated analyzer parameters and peripheral smear findings.

Exclusion criteria:

[1] Patients with incomplete or missing laboratory records.
[2] Patients with known hematological malignancies.
[3] Cases with recent blood transfusion

Data collection:

Information was retrieved from medical facility databases and laboratory logs. The parameters that were recorded are as follows:

[1] Hemoglobin (Hb) level
[2] Red blood cell (RBC) count
[3] Packed cell volume (PCV)
[4] Red cell indices: MCV, MCH, MCHC
[5] Red cell distribution width (RDW)
[6] Peripheral smear findings

Automated hematology analysis:

An automated hematology analyzer was used to test the blood samples. Anemia was categorized according to MCV values:

[1] Microcytic anemia (MCV <80 fL)
[2] Normocytic anemia (MCV 80–100 fL)
[3] Macrocytic anemia (MCV >100 fL)

In order to identify mixed anemia and measure fluctuation in RBC size, other metrics were used, such as RDW.

Peripheral smear examination:

Leishman stain was applied to peripheral blood smears that had been prepared according to conventional protocols. The following was assessed by means of microscopic analysis:

[1] RBC size (microcytic, normocytic, macrocytic)
[2] RBC morphology (anisocytosis, poikilocytosis)
[3] Chromasia (hypochromia, normochromia)
[4] Presence of abnormal cells or inclusions
[5] Based on morphological findings, anemia was classified into:
[6] Microcytic hypochromic anemia
[7] Normocytic normochromic anemia
[8] Macrocytic anemia
[9] Dimorphic anemia

Data analysis:

Diagnostic concordance was evaluated by comparing the results of automated hematology analyzers with those of peripheral smears. Analysis based on statistics comprised:

[1] Percentage agreement between the two methods
[2] Sensitivity and specificity of automated analyzer in detecting anemia subtypes
[3] Chi-square test for assessing statistical significance
[4] A p-value <0.05 was considered statistically significant.

Results:

This retrospective analysis comprised 120 pregnant women who were diagnosed with anemia. All patients got comprehensive data from peripheral smear testing and automated hematological analyzers. Population characteristics the patients' ages varied from 18 to 38, with 58.33% being within the 21–30 age brackets. The majority of patients were in the second and third trimesters (72.5%), which is consistent with the greater rates of anemia identification and increased physiological demand during this time. The most prevalent subtype of microcytic hypochromic anemia, which was seen in 56.67 percent of cases, according to peripheral smear testing, indicates that iron shortage is the main cause. Among the anemias that were less common, normocytic anemia accounted for 21.67%, macrocytic anemia for 8.33% and dimorphic anemia for 13.33%. Dimorphic anemia is a sign of many nutritional deficiencies, most notably an iron deficit together with a vitamin B12 or folate deficiency **(Table 1).** Microcytic anemia was found in 60% of cases, according to automated analyzer results, which were in agreement with peripheral smear results. Smear analysis revealed mixed or dimorphic patterns; however, the analyzer recorded a greater percentage of normocytic (28.33%) and macrocytic anemia (11.67%) **(Table 2)**. The results from the automated analyzer and the peripheral smear were consistent in 96 instances (or 80% of the total), whereas 24 cases (20%) were incorrectly categorized. Microcytic anemia showed the highest agreement (91.18%); Normocytic anemia showed moderate agreement (76.92%); Macrocytic anemia showed high agreement (80%); Dimorphic anemia had the lowest agreement (37.5%), indicating significant limitations of automated analyzers in detecting mixed anemia **(Table 3)**.

Table 1: Distribution of anemia types based on peripheral smear

Type of Anemia	Number (n=120)	Percentage (%)
Microcytic hypochromic	68	56.67%
Normocytic normochromic	26	21.67%
Macrocytic	10	8.33%
Dimorphic	16	13.33%
Total	120	100%

Table 2: Distribution of anemia types based on automated analyzer (MCV Classification)

Type of Anemia	Number (n=120)	Percentage (%)
Microcytic (MCV <80)	72	60.00%
Normocytic (80–100)	34	28.33%
Macrocytic (>100)	14	11.67%
Total	120	100%

Table 3: Comparison of peripheral smear and automated analyzer findings

Peripheral Smear Diagnosis	Automated Analyzer Correct	Misclassified	Total
Microcytic hypochromic	62	6	68
Normocytic normochromic	20	6	26
Macrocytic	8	2	10
Dimorphic	6	10	16
Total	96	24	120

Discussion:

Because of the risks it poses to both the mother and the unborn child, maternal anemia during pregnancy is an important issue in global public health, particularly in underdeveloped nations. Correctly diagnosing anemia is crucial for administering the right treatment. Using pregnant women as a sample, this research compared the diagnosis accuracy of automated hematology analyzers and peripheral smear testing for subtyping anemia. Microcytic hypochromic anemia was found in 56.67 percent of the cases in this investigation, according to the results of the peripheral smear. Consistent with other research, this finding confirms that iron deficiency is the primary cause of anemia during pregnancy **[2]**. This high incidence is caused in large part by nutritional inadequacies, inadequate food consumption and the increased physiological demands of pregnancy **[4]**. Microcytic anemia was indicated as the most common kind by the automated analyzer (60%), which aligns well with the results from the peripheral smear. Thomas and Chavan **[3]** also found that automated analyzers are quite sensitive to the presence of microcytic anemia. This is due to the fact that hemoglobin concentration and red cell size are consistently reflected by red cell indices like MCV and MCH. The number of instances of normocytic anemia was underestimated by automated analyzers (28.33%) compared to the number of cases found on peripheral smear (21.67%). The failure of automated methods to pick up on modest morphological differences or mixed impairments could explain this disparity **[5]**. Similar overestimation of normocytic anemia owing to limits of red cell indices alone has been documented in studies by Hussain and Frayez **[5]**. Peripheral smear detection rate for macrocytic anemia was 8.33%, whereas automated analyzer detection rate was 11.67%. Some instances were misclassified, even though there was reasonably high agreement. Smear testing is necessary to confirm macrocytosis observed by analyzers, as it may indicate reticulocytosis or an early nutritional deficit **[7]**. The peripheral smear results for dimorphic anemia, which made up 13.33% of all cases, had the lowest concordance with automated analyzer results at 37.5%. Misdiagnoses of normocytic or microcytic anemia occurred in the majority of these instances. This result agrees with research by Nain *et al.* that showed automated analyzers had a harder time picking up on mixed red cell populations **[8]**. It is therapeutically crucial to accurately diagnose dimorphic anemia during pregnancy because it is usually caused by a combination of iron deficiency and folate or vitamin B12 deficiency **[9]**. In this investigation, automated analyzers achieved an overall accuracy rate of 80% for diagnostics, with a sensitivity rate of 85% and a specificity rate of 88%. Consistent with previous research, our results show a sensitivity of 80-90% and a specificity of 85-95% **[10, 11]**. It seems that automated analyzers are dependable for screening purposes, given the high negative predictive value of 89.58 percent. A substantial association between the results of automated and peripheral smears is shown by the statistically significant p-value (<0.001). The disparities that have been found, however, show how vital peripheral smear screening is as an auxiliary diagnostic method. In cases when automated technologies fail to detect anisocytosis, poikilocytosis, or mixed cell populations, a peripheral smear may be used to directly observe the morphology of red blood cells **[12]**. This research also found that red cell distribution width (RDW) is a useful tool for diagnosing mixed anemia. Cases of dimorphic anemia were associated with elevated RDW values, indicating that RDW might be used as a supplementary criterion for the diagnosis of this condition **[13]**. It is necessary to evaluate RDW in combination with smear results, since RDW alone is insufficient. Although hematological analyzers have come a long way in recent years, they are still not able to fully replace human inspection in terms of diagnostic accuracy. Awaad *et al.* and Agarwal *et al.* emphasized that the most accurate diagnosis is achieved when automated analysis and peripheral smear testing are used together **[14, 15]**. Having access to modern diagnostic technologies is crucial, especially in situations with limited resources. The significance of combining the two diagnostic methods in everyday clinical practice is shown by the results of this investigation. Rapid and reliable screening is offered by automated analyzers, whereas conclusive morphological diagnosis is provided by peripheral smear testing. Collaboratively, they guarantee precise anemia categorization, allowing for prompt and suitable treatment.

Conclusion:

Automated hematology analyzers provide rapid preliminary anemia detection and classification in pregnant women with excellent sensitivity and specificity but have limitations in identifying mixed and dimorphic anemia. Peripheral smear remains the gold standard for morphological subclassification and should complement automated analysis for accurate diagnosis. These findings support a combined approach using automated hematology analysis and peripheral smear examination to optimize diagnostic accuracy and clinical outcomes.

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