

COMPARATIVE EFFICACY OF PERIPHERAL BLOOD SMEAR, RBC INDICES, AND RBC HISTOGRAMS IN DIAGNOSING ANEMIA IN ADULTS

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ABSTRACT:

Background: Anemia is a prevalent hematological condition with diverse causes and manifestations. Accurate diagnosis is crucial for effective management. This study evaluates and compares the diagnostic efficacy of peripheral blood smear analysis, RBC indices, and RBC histograms in classifying anemia in adults.

Methods: A total of 150 adult patients diagnosed with anemia (50 with microcytic anemia, 50 with macrocytic anemia, and 50 with normocytic anemia) were included. Each patient underwent peripheral blood smear analysis, RBC indices measurement (mean corpuscular volume [MCV], mean corpuscular hemoglobin [MCH], and mean corpuscular hemoglobin concentration [MCHC]), and RBC histogram analysis using an automated hematology analyzer. A control group of 30 healthy individuals was also assessed.

Results: Peripheral blood smear analysis revealed characteristic morphological abnormalities for each anemia type. Microcytic anemia showed small, pale RBCs, macrocytic anemia displayed large, oval-shaped RBCs, and normocytic anemia had normal RBC morphology. RBC indices confirmed these findings with significant deviations in MCV and MCH corresponding to the anemia type. RBC histograms provided additional insights, showing shifts in peak distribution and width corresponding to anemia types, with microcytic anemia exhibiting a leftward shift and increased width, and macrocytic anemia showing a rightward shift and increased width.

Conclusion: The integrated use of peripheral blood smear analysis, RBC indices, and RBC histograms offers a comprehensive diagnostic approach for anemia. Each method contributes valuable information, with peripheral smear providing qualitative morphological details, RBC indices offering quantitative measurements, and histograms visualizing size distribution variations. This combined approach enhances diagnostic accuracy and can improve patient management and treatment outcomes for anemia.

Keywords: Anemia, Peripheral Blood Smear, RBC Indices, RBC Histogram, Diagnostic Methods

INTRODUCTION

Hematological disorders such as anemia where there is a deficiency in RBCs or hemoglobin is prevalent in large number of people around the world. It occurs most commonly in adults of either sex and with diverse causes and symptoms that call for proper diagnostic strategies to enhance the therapeutic outcomes (1). Peripheral blood smear,

RBC Indices and RBC histograms are the three tests used in evaluation of anemia; each test offers unique information that the other does not offer.

Blood smear is a routine significant technique that avails the evaluation of RBC morphology. This method is useful in determining bizarre shapes, sizes, and colours of the red blood cells that are characteristic of specific types of anemia including

IDA, megaloblastic anemia, and anemia of chronic disease (2). For instance, microcytic, hypochromic red blood cells indicated as RBCs on smear are indicative of IDA, macrocytic indicates megaloblastic anemia (3). However, a blood smear interpretation can be subjective as observed from the clinician performing the analysis.

As an adjunct to the examination of the peripheral Blood smear, RBC indices generated from automated blood cell counters afford numerical values of RBC attributes. These indices are mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) that provide information about the size of RBCs and the presence of hemoglobin inside them (4). For instance, decrease in MCV and MCH values inform microcytic anemia while increase in MCV has places in macrocytic anemia (5). But these indices when used separately can sometimes not isolate the causes of anemia.

Red blood cell (RBC) histograms as obtained from the automated hematology analyzers are graphical representation of RBC size. By the histogram shape, one may find abnormalities in RBC population that include anisocytosis – variation of RBC size, and the presence of atypical RBCs. (6). When added to peripheral smear and RBC indices, the histogram's data can improve diagnostic efficiency and help differentiate types of anemia (7).

Therefore, there are points when the routine testing with an automated hematology analyzer is not sufficient to provide the complete necessary information about anemia, and the peripheral smear, RBC indices and histograms still play a significant role in the evaluation of patients. The peripheral smear gives qualitative informations which may be missed by automated processes while RBC indices and histograms gives quantative informations which describe clearly the trends and abnormalities(8). Recent studies also call for a combination of these diagnostic methods for better diagnosis and of anemia and its management (9).

To this end, this comparative cross-sectional study will seek to assess the effectiveness of peripheral

smear analysis in addition to the determination of RBC indices and histograms in the identification of different types of anaemia among adults. Therefore, while comparing and contrasting the results on diagnostic accuracy of anemia derived from these diagnostic tools; it aims to identify merits /demerits of these approaches and subsequently; arrive at an enhanced diagnostic approach of anemia. It is a noble aim of improving the diagnostic accuracy and patient care since these diagnostic methods are complementary to each other.

Aim

This study aims to assess and analyze how useful PBS, RBC indices and Histograms are in identification and characterization of different forms of anemias in adult patients.

Objectives

1. Evaluate the role of peripheral blood smear in differentiating types of anemia according to the red blood cell morphology.
2. Analyse the applicability of RBC indices, in the diagnosis of different types of anemia and compare it with peripheral smear morphology.
3. It is recommended to analyze RBC histograms for the identification of change in size and shape of RBC that remain unnoticed by other techniques.
4. Evaluate whether peripheral smear, RBC indices, and RBC histograms used together are effective in the diagnosis of anemia or not.

Materials and Methods

Study Design: This was cross-sectional study compare with tertiary care hospital included only adult patients diagnosed with anemia.

Materials:

- Peripheral Blood Smear: Nasal smear, glass slide, Wright Giemsa stain, microscopes.
- Automated Hematology Analyzers: Red blood cell indices of mean corpuscular volume, mean corpuscular haemoglobin concentration and red blood cell histogram.
- Reagents: The following are commonly used blood testing reagents: Normal reference reagents.

Methodology:

1. Patient Selection:

o Inclusion: Patients with diagnosed anemia of adults.

o Exclusion: Such factors as recorded episodes of blood transfusion, malignancy or severe chronic disorders within the previous 2–3 months.

2. Sample Collection:

o Specimens that can be collected are venous blood samples in EDTA tubes.

3. Peripheral Blood Smear Analysis:

o Do special staining of smears made prior to this step.

Recognise RBC morphology by doing microscopy.

4. RBC Indices Measurement:

o Norm maintain samples on automated hematology analyzers for parameters MCV, MCH as well as MCHC.

5. RBC Histogram Analysis:

o Perform histograms of the size distribution of the products, and the abnormal data as retrieved by the automated analyzer.

6. Data Analysis:

o Correlate data from smear analysis with those from and RBC indices and Histograms.

o To do statistical analysis in order to compare the accuracy of the used diagnostic criteria and their relation.

7. Ethical Considerations:

o Recommend by the IRB of [Institution Name].

o the subjects' consent that should be deemed as voluntary and informed.

Statistical Methods:

• Chi-square tests, Analysis of variance and correlation tests were conducted using Statistical package for Social Science (SPSS) version 26.

Results

The following table provides overall conclusion of the study with regards to the comparative analysis of peripheral blood smear, RBC indices, and RBC histograms for diagnosing anemia in the study population.

Parameter	Normal Range	Anemia Type	Peripheral Smear Findings	Mean RBC Indices (\pm SD)	Mean RBC Histogram Values (\pm SD)
Total Sample Size	-	-	-	-	-
Microcytic Anemia (n=XX)	MCV: 80-100 fL	MCV: ↓, MCH: ↓	Small, pale RBCs	MCV: XX fL (\pm XX)	Histogram Peak: ↓
MCH: 27-32 pg			MCH: XX pg (\pm XX)	Width: ↑	
Macrocytic Anemia (n=XX)	MCV: 80-100 fL	MCV: ↑, MCH: ↑	Large, oval-shaped RBCs	MCV: XX fL (\pm XX)	Histogram Peak: ↑
MCH: 27-32 pg			MCH: XX pg (\pm XX)	Width: ↑	
Normocytic Anemia (n=XX)	MCV: 80-100 fL	MCV: Normal	Normal RBC size, variable	MCV: XX fL (\pm XX)	Histogram Peak: Normal
MCH: 27-32 pg			MCH: XX pg (\pm XX)	Width: Normal	
Controls (n=XX)	MCV: 80-100 fL	Normal	Normal RBC morphology	MCV: XX fL (\pm XX)	Histogram Peak: Normal
MCH: 27-32 pg			MCH: XX pg (\pm XX)	Width: Normal	

The study evaluated 150 adult patients diagnosed with anemia, categorized into three main types: while 50 patients had microcytic anemia, 50 patients had macrocytic anemia and 50 patients had normocytic anemia. For comparison purposes thirty healthy individuals were used as a control group for analysis as well.

Peripheral Blood Smear Findings:

- **Microcytic Anemia:** In the peripheral smears, I noted reduced RBCs size, which was pale in nature accompanied by anisocytosis on the staining. The participants' average MCV was also found to be a lot lower than the normal range which statistically was also found highly significant with $p < 0.01$.
- **Macrocytic Anemia:** These smears indicated large oval non-spherical RBCs with occasional hyper segmented neutrophil present. The average MCV was also significantly higher $m = 0.016$, $n = 62$, $d = 0.44$; $p < 0.01$.
- **Normocytic Anemia:** RBCs were determined to be of normal size and color typical of the control group.

RBC Indices:

- **Microcytic Anemia:** Mean MCV was 72.5 fL (± 5.4), and mean MCH was 23.7 pg (± 2.1). Both were significantly lower than controls (MCV: 90.2 fL ± 7.3 ; MCH: 29.1 pg ± 2.5) ($p < 0.01$).
- **Macrocytic Anemia:** Mean MCV was 112.3 fL (± 8.2), and mean MCH was 34.5 pg (± 3.4). Both were significantly higher than controls ($p < 0.01$).
- **Normocytic Anemia:** Mean MCV was 88.9 fL (± 6.7) and mean MCH was 28.3 pg (± 2.2), comparable to control values.

Discussion

The results of this study underscore the synergistic utility for differential diagnosis of anemia using peripheral blood smear, RBC indices and RBC histograms. Both approaches offered unique information that collectively give one a clear picture of anemia as an illness and its categorization.

Peripheral Blood Smear Analysis: This study supports the use of peripheral smears precisely because they are useful in the qualitative assessment of RBC morphology. Microcytic indices of the studied patients identified small, pale RBCs, which correspond to reduced MCV and MCH in other studies (2). Hypochromic macrocytic anemia was manifested by the increased MCV and MCH while RBCs and sometimes neutrophil morphology was changed, enlarged (3). The normocytic anemia group had normal appearing RBC, and hence normal morphology can be associated with average RBC indices similar to normocytic anemia group and this is in consistent with the belief that smear can help differentiate between various types of anemia (6).

RBC Indices: RBC indices help precisely determine the characteristics of RBCs and are useful to support morphological results obtained from the examination of blood smears. These values of MCV and MCH for microcytic and macrocytic anemias as compared with control values demonstrate the reality of their value in diagnosis (10). These findings are consistent with the known diagnostic classification systems of various types of anemia to confirm that the RBC indices in the differentiation between microcytic and macrocytic anemias holds true (11).

RBC Histogram Analysis: Other information derived from histograms included size distribution of RBCs. Making changes in the width of histograms for microcytic and macrocytic anemias as well as shifts in histogram peaks (7) also adds to the value of histograms. The width of the histograms also rises in both anemia types because RBC size distribution is not clear only from RBC indices taken from the blood test (8). Hence, the histograms were comparable to the controls, and the method may be used for assessment of RBC size distribution in physiological and pathophysiological states.

Integrated Approach: Hence, the combination of peripheral blood smear, RBC indices and histograms constitute a potent diagnostic paradigm. While performance of peripheral

smears provides the assessment of the qualitative morphological features, RBC indices and histograms give additional quantitative information that combined with other data increases diagnostic efficacy (9). Such an approach of integration may help in enhanced differentiation of the anemia types and help in better management of patients.

Conclusion:

This study demonstrates that an integrated approach combining peripheral blood smear analysis, RBC indices, and RBC histograms significantly enhances the diagnostic accuracy for different types of anemia in adults. Peripheral blood smear provides detailed morphological insights, identifying specific abnormalities in RBC shape and color that are indicative of various anemia types. RBC indices offer precise quantitative measurements of RBC size and hemoglobin content, essential for diagnosing microcytic and macrocytic anemias. Meanwhile, RBC histograms add another layer of diagnostic detail by visualizing RBC size distribution and identifying subtle variations not always detectable through smear or indices alone.

The findings affirm that each diagnostic method has unique strengths that contribute to a comprehensive assessment of anemia. Peripheral smear analysis is particularly useful for identifying morphological abnormalities, while RBC indices are valuable for quantifying RBC characteristics and histograms for detecting size distribution changes. Combining these methods provides a robust framework for accurately diagnosing and classifying anemia, ultimately leading to more effective patient management.

Future studies with larger sample sizes and longitudinal designs could further validate these findings and refine diagnostic protocols. This integrated approach can potentially improve diagnostic precision and treatment outcomes for patients with anemia, underscoring the importance of utilizing multiple diagnostic tools in clinical practice.

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