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Abstract

Anemia is one of the most common hematological disorders encountered in clinical practice and remains a major public health concern, particularly in developing countries. Accurate diagnosis and proper morphological classification are essential for effective management. This study was conducted to assess the correlation between red cell indices obtained from an automated hematology analyzer and peripheral smear findings in patients diagnosed with anemia.

A hospital-based cross-sectional observational study was carried out over a period of six months, including 80 anemic patients aged 18 years and above. Complete Blood Count analysis was performed to record hemoglobin levels and red cell indices, including Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), and Red Cell Distribution Width (RDW). Peripheral blood smears were prepared using Leishman stain and examined microscopically for morphological evaluation.

Among the study population, females constituted 60% of cases, and the highest prevalence was observed in the 18–30 years age group. Moderate anemia was the most common severity pattern (47.5%). Based on red cell indices, microcytic anemia was predominant (57.5%), which was confirmed by peripheral smear examination showing microcytic hypochromic morphology in 55% of cases. Elevated RDW values correlated strongly with anisocytosis observed on smear examination. A high correlation accuracy of 92.5% was observed between MCV-based classification and peripheral smear findings, which was statistically significant ($p < 0.05$).

The study concludes that red cell indices, particularly MCV and RDW, are reliable for the initial morphological classification of anemia. However, peripheral smear examination remains essential for confirmation and identification of additional morphological abnormalities. A combined approach using automated hematology parameters and peripheral smear evaluation provides a more accurate and comprehensive method for diagnosing and classifying anemia in routine laboratory practice.

Keywords: Anemia, Red Cell Indices, MCV, RDW, Peripheral Smear, Morphological Classification, Complete Blood Count.

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Introduction

Anemia is among hematologic diseases most frequently met in clinical situations. It is defined as a decrease in the level of hemoglobin, the hematocrit, or the red blood cells in blood under the normal range of blood components in relation to age and sex (Liem et al., 2019). Hemoglobin is essential for the transportation of oxygen to the various parts of the body, and when there is a reduction, the tissues will lack oxygen causing related symptoms such as fatigue, pale skin, breathlessness, dizziness, and a decrease in work capacity. In developing countries such as India, anemia is a major health issue of the population attributed to nutritional deficiencies, parasitic infections, chronic diseases and hereditary disorders (Gallagher, 2022).

Laboratory Methods of Diagnosing Anemia The primary approaches to diagnosis of anemia are complete blood count parameters and the morphology of red blood cells. Automated hematology analyzers give quantitative information about the blood in red cell indices and peripheral smear examination gives the qualitative information by morphology (Garca et al., 2023). Correlating these two approaches aids in increasing the diagnostic accuracy and the identification of the underlying cause of the anemia.

2.1 Overview of Anemia
Anemia is a result of decreased oxygen-carrying capacity of the blood because of decreased hemoglobin levels or abnormal red blood cells. The World Health Organization (WHO) definition of anaemia is based on the proportion of haemoglobin in blood in the body and is defined as haemoglobin less than 13 g/dL in adult males, less than 12 g/dL in adult females and less than 11 g/dL in pregnant women (Akbarpour et al., 2022).

Globally, the most common form of anemia is iron deficiency anemia, especially in women and children. Other causes are vitamin B12 deficiency, folate deficiency, chronic inflammatory conditions, bone marrow disorders, hemolysis and genetic abnormalities (Mwafy et al., 2022). It is important to diagnose early because untreated anemia can cause serious complications like impaired cognitive development, low immunity and strain on the cardiovascular system.

2.2 Classification of Anemia
Anemia may be categorized in various ways:
1. Morphological Classification (Based on the size of the red cell and the quantity of hemoglobin per cell)

- Microcytic hypochromic anemia
- Anemia Normocytic normochromic
- Macrocytic anemia
- This classification is primarily defined on the red cell indices such as MCV or MCHC.

2. Etiological Classification (According to Cause)
The nutritional anemias (iron, vitamin B12, folate deficiency)

- Hemolytic anemia

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- Aplastic anemia
- Anemia of chronic disease
- Hemoglobinopathies

Morphological classification is very helpful in routine laboratory practice as it gives a clue to the underlying cause.

- Role of Red Cell Indices (MCV, MCH, MCHC, RDW) 2.3
- Red concern these are calculated parameters offered by automated hematology analyzers throughout total blood count testing.
- Enteroviruses (EV): Respiratory infections are the leading cause of cough. It is useful in classifying the anemias as microcytic, normocytic or macrocytic.
- MCH (Mean Corpuscular Hemoglobin): Shows the average RBC consisting of hemoglobin.
- MCHC (Mean Corpuscular Hemoglobin Concentration): Shows the concentration of hemoglobin of a volume of packed red cells.
- RDW (Red Cell Distribution Width): Determines the difference in red blood cell size and is useful to distinguish varieties of anemia.

These indices are useful as they provide rapid and objective information. For instance, low MCV indicates iron deficiency anemia and high MCV can be a symptom of a vitamin B12 or folate deficiency. Increased RDW's are commonly observed in early nutritional anemia.

2.4 Importance of Peripheral Smear Examination

Peripheral smear examination is one of the basic tools of hematology. A well prepared and correctly stained smear enables one to see the morphology of red blood cell directly under the microscope.

It helps identify:

- Differences in size (anisocytosis)
- Variation in shape (poikilocytosis)
- Hypochromia
- Target cells
- Spherocytes
- Macro-ovalocytes
- Polychromasia

While automated analyzers provide numerical data, they cannot completely replace the morphological assessment. Peripheral smear examination is used to confirm findings of analyzer and find errors that machines may miss.

2.5 Rationale of the Study

Although there is widespread use of automated hematology analyzers, using only red cell indices for diagnosis can occasionally result in the diagnosis not being complete. Peripheral smear examination is important for morphological confirmation and to see additional abnormalities.

Correlating red cell indices with peripheral smear findings leads to better diagnosis, appropriate classification of anemia, and proper management of the patient. In resource-limited settings, this is a cost-effective and practical approach that combines.

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Therefore, this study is focusing to assess the correlation of red cell indices with peripheral smears in patients admitted with anemia which will assess the diagnostic reliability in anemia and its clinical significance.

Review of Literature

Anemia has been the subject of a great deal of research because of its high prevalence worldwide and its importance as a clinical issue. Numerous researchers have stressed the benefits of automated hematological parameters as well as peripheral smear examination in anemia diagnosis and classification (Brugnara, 2015).

Several epidemiological studies have shown that the most common form of anemia in the world is iron deficiency anemia, especially among women of reproductive age and children. Studies conducted in Indian hospital settings have demonstrated a high prevalence of microcytic hypochromic anemia which is predominantly linked to nutritional deficiencies. These results demonstrate the necessity for an accurate and early laboratory diagnosis (Bashir & Khan, 2013).

Research into red cell indices has proved them to be useful in the preliminary classification of anaemia. MCV has been generally accepted as a major parameter for the morphological division into microcytic, normocytic and macro (Mettananda et al., 2020). Studies have shown that there is a strong correlation between low MCV values and iron deficiency anemia, and high MCV values are usually associated with vitamin B12 and folate deficiency. MCH and MCHC have also been found useful in identification of hypochromic states. Increased RDW has been described as an early sign of developing nutritional anaemia, even before significant changes in the level of haemoglobin have been observed.

Comparative studies between automated hematology analyzers and peripheral smear examination have shown a significant correlation between red cell indices and red cell methodological findings. Many researchers noticed that a low MCV and MCH value relates very well with microcytic hypochromic blood pictures on smear. Similarly, the presence of macro made on peripheral smear (highly correlated with high MCV) as well as d exists as macrocytosis high MCV is also detected on peripheral smear.

However, literature also goes on to indicate certain limitations of actually relying only on automated indices. Some studies have reported cases of appearance of red cell indices within normal limits despite the presence of evident morphological abnormalities on smear examination. Conditions such as dimorphic anemia, early iron deficiency and mixed nutritional deficiencies may not be clearly differentiated by indices alone. In such situations peripheral smear examination plays an important confirmatory role.

Studies evaluating RDW has shown its diagnostic importance in differentiating iron deficiency anemia and anemia of

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chronic disease. Increased RDW should commonly be seen in iron deficiency anemia, while it is frequently normal in anemia of chronic disease(Umar, 2017). This parameter has been emphasized as a simple and effective way in a cost-effective laboratory practice.

Research has also stressed the value of good laboratory quality control in the hematology laboratory. Proper calibration of automated analyzers and standard smear preparation techniques have major benefits for more accurate diagnosis(Stewart et al., 1987). Many authors recommend that the abnormal red cell indices should always be correlated with the findings of peripheral smear staining in an attempt to avoid misinterpretation.

All in all, the current literature supports the use of red cell indices as well as peripheral smear examination in the case of anemia supplementation. Although automated analyzers have a very fast response and are able to provide quantitative data, morphological assessment is needed for complete diagnosis(Garca et al., 2023b). These findings are the basis of conducting the present study to further assess the correlation between the red cell indices and peripheral smear findings in patients with anemia.

Aims and Objectives

4.1 Aim

To assess the correlation between red cell indexes and peripheral smear results in diagnosing and morphologic classification of anemia.

4.2 Objectives

1. To determine hemoglobin and red cell indices (MCV, MCH, MCHC, RDW) of patients diagnosed as anemic.
2. To examine peripheral blood smears of the anemic patients and classify anemias on the basis of morphological patterns.
3. To be able to correlate red cell indices with corresponding peripheral smear findings.
4. To examine the diagnostic value of red cell indices in classifying morphological types of anemia.
5. To determine the utility of using combined automated hematology parameters and the peripheral smear examination in routine laboratory practice.

5. Materials and Methods

5.1 Study Design

This study is going to be a hospital based cross-sectional observational study. The objective is to assess the correlation between the values obtained for red cell indexes using an automated hematology analyzer and the changes observed under the peripheral smear in patients diagnosed with anemia. All samples will be analyzed at one single time without intervening.

5.2 Study Setting

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The research will be conducted in the Department of Hematology / Clinical Laboratory of a tertiary care hospital. Blood samples will be taken from patients attending in the outpatient department and inpatient wards whose complete blood counting testing is recommended as part of routine clinical evaluation.

5.3 Study Duration

The study will be carried out over a period of 6 months. This duration will, apart from patient selection, sample collection, laboratory analysis, data compilation, statistical analysis and report preparation.

5.4 Sample Size

A total of 80 patients diagnosed with anaemia will be included in the study. The size of the sample is decided based on feasibility and previous similar laboratory based studies to be able to ensure good statistical correlation.

5.5 Inclusion Criteria

- Patients of both the sexes diagnosed with anemia based on the normal range of hemoglobin.
- The selection criteria for this clinical trial were as follows: - Patients of age 18 years and older.
- Patients who give their informed consent to be a part of the study.
- Blood samples taken in blood collection vials in anticoagulated (EDTA) blood for CBC, peripheral smear examination.

5.6 Exclusion Criteria

- For Example: - Patient who has taken blood transfusion within last three months.
- Patients who are undergoing chemotherapy or radiotherapy
- Dysfunctional fistula: - Rationale: - Females who have a history of fetal or neonatal cyanotic heart disease - Females who have known hematological malignancies.
- Clotted, Hemolyzed or Improperly Collected Blood Samples

Previously, there were very few hospitals that could serve as temporary homes for the sick during World War II, as no one realized that large numbers of people could be infected with the virus and die at a much faster rate than the virus could be destroyed.

5.7 Sampling Collecting Procedure

After obtaining informed consent, approximately 2-3 mL of venous blood will be collected using aseptic precautions using a sterile disposable syringe or vacutainer system. The blood sample will be transferred into one of the EDF (ethylenediaminotetraacetic acid)Anticulated blood tubes and will be mixed gently to avoid forming blood clots.

Patient details such as age, gender and hemoglobin level will be entered in a structured data collection proforma. The sample inside the hospital will have the sample processed within 2 hours of its collection to ensure accuracy of the hematological parameters.

5.8 Laboratory Investigations

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All samples will undergo Complete Blood Count analysis followed by peripheral smear examination.

5.8.1 Complete Blood Count (CBC)

CBC will be performed using an automated hematology analyzer as per manufacturer guidelines. The analyzer will be calibrated and quality control procedures will be followed before processing patient samples.

Parameters recorded will include:

- Hemoglobin (Hb)
- Red Blood Cell count (RBC)
- Hematocrit (HCT)
- Mean Corpuscular Volume (MCV)
- Mean Corpuscular Hemoglobin (MCH)
- Mean Corpuscular Hemoglobin Concentration (MCHC)
- Red Cell Distribution Width (RDW)

5.8.2 Red Cell Indices Evaluation

Red cell indices including MCV, MCH, MCHC, and RDW will be analyzed to classify anemia morphologically as:

- Microcytic (MCV < 80 fL)
- Normocytic (MCV 80–100 fL)
- Macrocytic (MCV > 100 fL)

MCH and MCHC values will be used to determine hypochromic or normochromic status. RDW values will be evaluated to assess variation in red blood cell size. These indices will then be correlated with peripheral smear findings.

5.8.3 Peripheral Smear Preparation and Staining

A thin blood smear will be prepared on a clean glass slide using the wedge method. The smear will be air-dried and stained using Leishman stain.

The stained smear will be examined under a microscope using low power and oil immersion objectives. Red blood cell morphology will be assessed for:

- Cell size (microcytosis, macrocytosis)
- Hemoglobin content (hypochromia)
- Variation in size (anisocytosis)
- Variation in shape (poikilocytosis)
- Presence of abnormal cells

Based on morphological findings, anemia will be classified as microcytic hypochromic, normocytic normochromic, or macrocytic.

5.9 Statistical Analysis

The collected data will be entered into Microsoft Excel and analyzed using SPSS software.

- Descriptive statistics such as mean and standard deviation will be calculated for quantitative variables.
- Frequency and percentage will be used for categorical variables.
- Correlation between red cell indices and peripheral smear findings will be assessed using

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Pearson's correlation test or Chi-square test where applicable.

- A p-value less than 0.05 will be considered statistically significant.

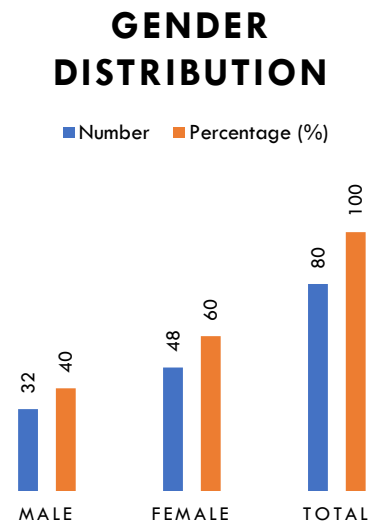
6. Results

A total of **80 patients diagnosed with anemia** were included in the study. The findings are presented below with detailed interpretation.

6.1 Demographic Distribution

Table 1: Gender Distribution (n = 80)

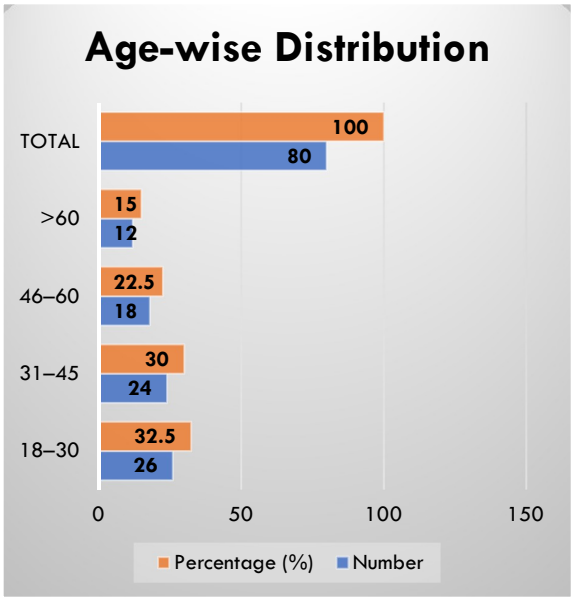
Gender	Number	Percentage (%)
Male	32	40
Female	48	60
Total	**80**	**100**



Interpretation:
Female patients constituted the majority of the study population (60%). This higher prevalence among females may be attributed to nutritional deficiencies, menstrual blood loss, and increased physiological demands.

Table 2: Age-wise Distribution (n = 80)

Age Group (Years)	Number	Percentage (%)
18–30	26	32.5
31–45	24	30
46–60	18	22.5
>60	12	15
Total	**80**	**100**



Interpretation:
The highest number of anemic patients belonged to the 18–30 years age group (32.5%). This suggests that young adults, particularly women of reproductive age, are more vulnerable to anemia.

6.2 Hemoglobin Distribution

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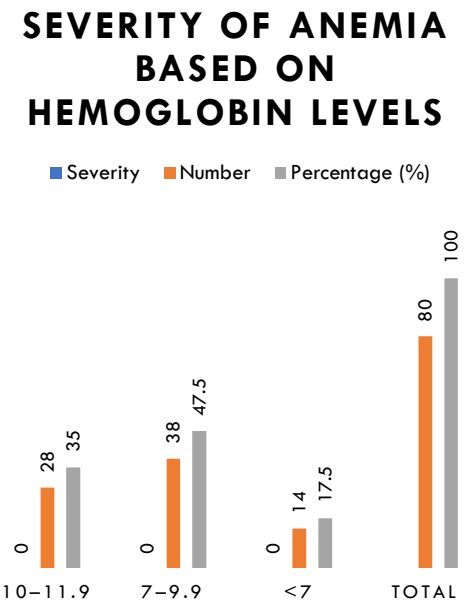
Table 3: Severity of Anemia Based on Hemoglobin Levels

Hemoglobin (g/dL)	Severity	Number	Percentage (%)
10–11.9	Mild	28	35
7–9.9	Moderate	38	47.5
<7	Severe	14	17.5
Total		**80**	**100**

Mean Hemoglobin Level: **8.9 ± 1.8 g/dL**



Interpretation:
Moderate anemia was the most common presentation (47.5%). Severe anemia was observed in 17.5% of cases, indicating the need for early detection and appropriate management.

6.3 Distribution of Red Cell Indices

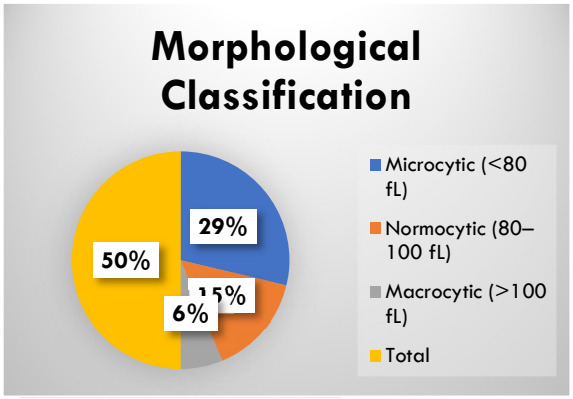
Table 4: Mean Red Cell Indices (n = 80)

Parameter	Mean ± SD
MCV (fL)	78.4 ± 9.6
MCH (pg)	25.1 ± 3.2
MCHC (g/dL)	30.8 ± 2.4
RDW (%)	16.9 ± 2.8

Interpretation:
The mean MCV value (78.4 fL) indicates a tendency toward microcytosis in the study population. Elevated RDW (mean 16.9%) suggests significant variation in red cell size, commonly seen in nutritional anemia.

Table 5: Morphological Classification Based on MCV

MCV Category	Number	Percentage (%)
Microcytic (<80 fL)	46	57.5
Normocytic (80–100 fL)	24	30
Macrocytic (>100 fL)	10	12.5
Total	**80**	**100**



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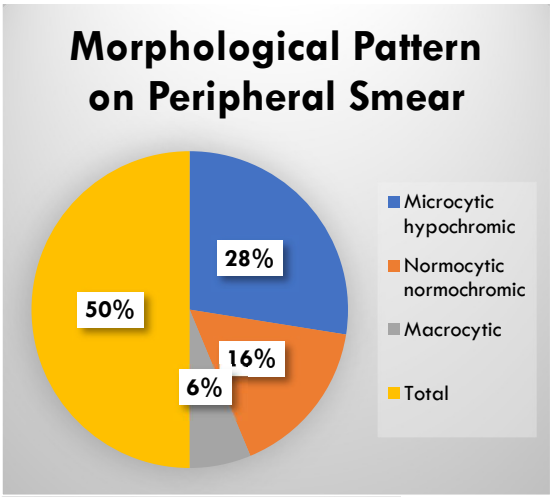
Interpretation:
Microcytic anemia was the predominant type (57.5%), indicating iron deficiency as a likely major cause in the study group.

6.4 Peripheral Smear Findings

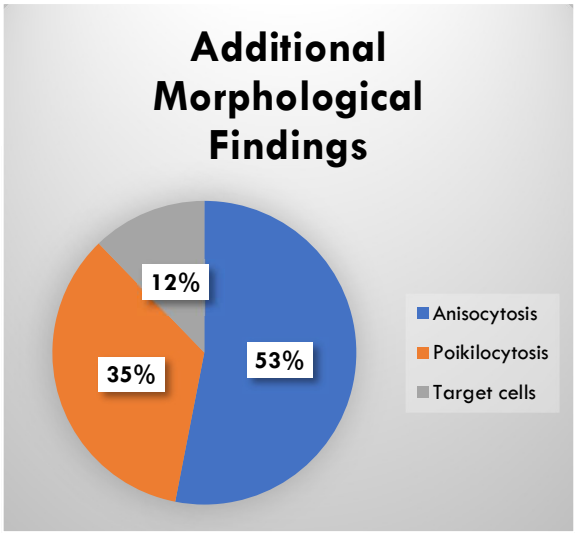
Table 6: Morphological Pattern on Peripheral Smear

Smear Pattern	Number	Percentage (%)
Microcytic hypochromic	44	55
Normocytic normochromic	26	32.5
Macrocytic	10	12.5
Total	**80**	**100**



Additional Morphological Findings:

Feature	Number	Percentage (%)
Anisocytosis	52	65
Poikilocytosis	34	42.5
Target cells	12	15



Interpretation:
Peripheral smear examination confirmed that microcytic hypochromic anemia was the most common pattern (55%). The presence of anisocytosis in 65% of cases correlates with increased RDW values observed in CBC analysis.

6.5 Correlation Between Red Cell Indices and Smear Findings

Table 7: Correlation of MCV with Peripheral Smear Findings

MCV Category	Matching Smear Pattern	Number of Matching Cases
Microcytic (46 cases)	Microcytic hypochromic	42
Normocytic (24 cases)	Normocytic normochromic	22
Macrocytic (10 cases)	Macrocytic	10

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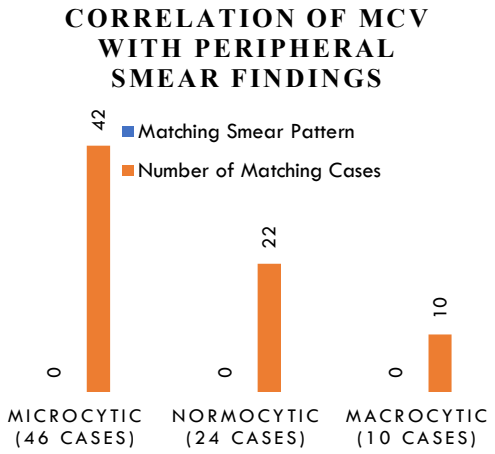

- Total Matching Cases: **74 out of 80**
- Correlation Accuracy: **92.5%**
- Chi-square test showed statistically significant association ($p < 0.05$).

Interpretation:
A strong correlation (92.5%) was observed between red cell indices and peripheral smear findings. Most cases classified as microcytic by MCV showed corresponding microcytic hypochromic morphology on smear. Minor mismatches were seen in 6 cases, possibly due to early or mixed anemia.

6.6 Graphical Representation (Description for Project)

The following graphical presentations were prepared for visual interpretation:

- Bar diagram showing gender distribution
- Pie chart representing severity of anemia
- Bar diagram showing distribution of MCV categories
- Comparative bar diagram showing correlation between MCV classification and smear findings

Overall Interpretation of Results

The study demonstrates that microcytic hypochromic anemia is the most prevalent morphological type among anemic patients. Red cell indices, particularly MCV and RDW, showed strong agreement with peripheral smear findings. The statistically significant correlation confirms that automated hematology parameters are reliable indicators for initial classification of anemia.

However, peripheral smear examination remains essential for confirmation and identification of additional morphological abnormalities.

7. Discussion

The current research was aimed at testing the relationship between red cell indices and the results of peripheral smear analysis in diagnosing anemia in 80 patients. The results indicated that there was a good relationship between automated hematology parameters and morphological analysis.

In the current research, women formed the largest number of cases (60%). This finding is in line with the established fact of high prevalence of anemia in women especially within the reproductive age due to blood loss during menstruation,

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nutritional deficiencies and other physiological demands. The maximum cases were recorded in 1830 years age group, which also proves that young adults are susceptible to nutritional anemia.

The most frequent severity pattern (47.5%), and mild anemia (35%), represented moderate anemia. We found cases of severe anemia (17.5). These results suggest that a large percentage of patients appear in the moderate period, which is why it is necessary to screen people on a regular basis and conduct lab tests at the earliest possible stages.

According to red cell indices, the most common type was microcytic anemia (57.5%). The average MCV of 78.4 fL was also in favor of the fact that there was more microcytosis. Peripheral smear examination was done to confirm that 55 percent of the patients demonstrated microcytic hypochromic pattern. This gives iron deficiency a strong indication as the most prevalent underlying cause of the study population. The high level of RDW (mean 16.9%), and anisocytosis in 65 percent of the cases also confirm the diagnosis of nutritional anemia.

MCV classification had a good correlation of 92.5% in comparison to the results of peripheral smear. Majority of the patients that were classified as microcytic in terms of MCV had the microcytic hypochromic morphology corresponding to the smear. In the same manner, morphological confirmation of macrocytic cases detected based on high MCV values was done. Very few cases which were not matched were noticed which can be explained by the fact that there were early cases of anemia or mixed cases of deficiencies.

The statistically significant relationship ($p < 0.05$) proves that red cell indices are effective predictors of the preliminary diagnosis of anemia. Nonetheless, the peripheral smear analysis offered further morphological information like anisocytosis, poikilocytosis and the target cells that are not completely accessible when using automated analyzers.

These results highlight the fact that automated hematology analyzers offer quick and objective quantitative results, but peripheral smear analysis is needed as a part of the complete assessment. The combined method is more accurate in the diagnosis and guarantees proper clinical treatment.

8. Conclusion

The current research has arrived at the conclusion that the most prevalent morphological anemia among the anemic patients of the study population is the microcytic hypochromic anemia. There is a close correlation between red cell indices (especially MCV and RDW) and the peripheral smear results.

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It was found that there was statistically significant association of automated red cell parameters with morphological classification of anemia. This suggests that red cell indices are efficacious to preliminary screening and identification of anemia.

Nevertheless, peripheral smear analysis cannot be replaced by any other technique to confirm and identify other morphological abnormalities. Consequently, the overall assessment of the red cell indices and the peripheral smear results is suggested to be used in determining the precise diagnosis and managing anemia in a regular laboratory.

9. Recommendations

In accordance with the results of the current research, the following recommendations can be offered:

1. The automated hematology analyzers should be used as a preliminary screening tool to make morphological classification of anemia.
2. Peripheral smear examination is advised to be regularly carried out in every anemic patient and during abnormal red cell indices.
3. Automated parameters and smear findings should be interpreted together in the normal lab practice in order to enhance accuracy of diagnosis.
4. There is a need to promote early screening especially to the female population and young adults to alleviate the burden of the nutritional anemia.
5. To have reliable results, laboratories should have strict internal quality control measures on both automated analyzers as well as smear preparation methods.
6. Additional research involving more samples and biochemical data (i.e., serum ferritin and vitamin B12 level) should be considered in order to define etiological diagnosis more narrowly.

10. Limitations of the Study

1. The sample size used in the study is quite small (80 patients), and this could restrict the extrapolation of the findings.
2. The research was institutionalized in the hospital and it might not mirror the actual prevalence trend in the general population.
3. No investigation of the biochemical, like serum iron, ferritin, vitamin B12 and folate levels was involved and this restricts the confirmation of the precise etiology of anemia.
4. The research was primarily based on morphological classification and excluded molecular and sophisticated diagnostic methods.
5. Lack of a broad spectrum of anemia cases might be due to short length of study.

11. Summary

Anemia is one of the most common hematological disorders seen in clinical practice and represents a serious public health problem especially in developing countries. Acc soprano e bass soon as

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good as solute and with an out standard diagonal and cinema arteria diagnose helps avert troubles and to get treatment adequately. The present study was carried out to assess the correlation between the red cell indices with automated hematology analyzers and peripheral smear findings in the diagnosis of anemia.

This hospital-based cross section study involved 80 patients who were diagnosed with anaemia. Complete Blood Count analysis was carried out by an automated hematology analyzer and red cell indices namely MCV, MCH, MCHC, and RDW were recorded. Peripheral blood smears were prepared, stained and microscopically examined for the morphology of red blood cells.

The majority of patients were females (60%) and the peak number of cases were of 18 - 30 years age group. Moderate anemia was the most common pattern of severity size noted. Based on red cells indexes, microcytic anemia predominated and this finding was substantiated by peripheral smear examination which revealed that majority of them having microcytic hypochromic morphology.

A strong correlation of 92.5% was studied between the red cell indexes and findings from peripheral smear and this correlation has been statistically significant ($p < 0.05$). Elevated RDW values showed a good correlation to occurrence of anisocytosis on smear examination. These results suggest that red cell indices are reliable in the initial morphological classification of anemia.

However, peripheral smear examination gave more morphological information that cannot be fully assessed by automated analysers alone. Therefore, the study concludes that red cell indices and peripheral smear results should be evaluated together to represent a more accurate and comprehensive way of diagnosing and classifying anemia.

The results of this study vouch for using both the automated values of hematological parameters and the microscopic examination in clinical laboratory, to assure the perfect diagnosis and patient management.

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