

Research Article

Morphological Spectrum of Microcytic Hypochromic Anemia and Correlation with Iron Profile and Hemoglobin Electrophoresis

Dr. Bapurao Kamaji Pawar¹, Dr. Chandra Bhushan², Dr. Prashant Kumar³

¹Assistant Professor from IQ City Medical College, Durgapur, WB.

²Assistant Professor from Noida International Institute of Medical Sciences, Noida, UP.

³Assistant Professor from Al-Falah School of Medical Sciences and Research Centre, Faridabad, HR.

Received: 17.07.22, Revised: 20.08.22, Accepted: 11.09.22

ABSTRACT

Background: Microcytic hypochromic anemia is commonly encountered in hematological practice and is most frequently caused by iron deficiency anemia (IDA) and thalassemia. Differentiating these conditions is important because their management differs considerably. This study evaluated the morphological spectrum of microcytic hypochromic anemia and correlated hematological findings with iron profile and hemoglobin electrophoresis/HPLC.

Methods: This cross-sectional observational study included 130 patients with microcytic hypochromic anemia. Complete blood count, red-cell indices, peripheral blood smear morphology, serum ferritin, serum iron, TIBC, transferrin saturation, and hemoglobin electrophoresis/HPLC were assessed. The Mentzer index was calculated, and correlations between hematological and iron parameters were evaluated.

Results: The mean age was 34.8 ± 15.2 years, and 58.5% of patients were female. IDA was the most common diagnosis (60.0%), followed by β -thalassemia trait (18.5%), anemia of chronic disease/inflammation (9.2%), and combined IDA with β -thalassemia trait (6.2%). Patients with β -thalassemia trait had a higher RBC count (5.61 vs 4.18 million/ μ L; $p < 0.001$), lower MCV (62.4 vs 68.9 fL; $p < 0.001$), and lower RDW (15.0% vs 19.1%; $p < 0.001$) than those with IDA. Serum ferritin was significantly lower in IDA, whereas HbA₂ was significantly higher in β -thalassemia trait (5.2% vs 2.5%; $p < 0.001$). A Mentzer index < 13 demonstrated 87.5% sensitivity, 89.7% specificity, and an AUC of 0.91 for identifying β -thalassemia trait.

Conclusion: IDA was the predominant cause of microcytic hypochromic anemia. Integration of peripheral smear morphology, RBC indices, iron profile, and hemoglobin analysis provides a reliable approach for distinguishing IDA from β -thalassemia trait.

Keywords: Microcytic Hypochromic Anemia, Iron Deficiency Anemia, β -Thalassemia Trait, Serum Ferritin, Hemoglobin Electrophoresis, Hplc, Mentzer Index.

INTRODUCTION

Anemia is one of the most common hematological disorders worldwide and remains a major public health problem, particularly in low- and middle-income countries. It is characterized by a reduction in hemoglobin concentration below the normal reference range for age, sex, and physiological status.[1] Among the various morphological patterns of anemia, microcytic hypochromic anemia is frequently encountered in routine hematological practice and is characterized by reduced mean corpuscular volume (MCV), decreased mean corpuscular hemoglobin (MCH), and varying degrees of hypochromia on peripheral blood smear examination.[2] Microcytic hypochromic anemia results primarily from impaired hemoglobin synthesis. The major causes include iron deficiency anemia (IDA), thalassemia, anemia of chronic

disease/inflammation, and sideroblastic anemia.[3] Although these conditions may share similar morphological features, their underlying pathophysiology, laboratory findings, clinical implications, and management differ considerably. Accurate identification of the etiology is therefore essential to avoid inappropriate treatment and to guide further investigation.[4]

Iron deficiency is considered the most common nutritional cause of anemia worldwide. It develops when body iron stores become insufficient to meet the requirements for normal erythropoiesis and hemoglobin synthesis.[5] Progressive iron depletion results in reduced hemoglobinization of red blood cells, producing microcytosis and hypochromia. Peripheral blood smears typically demonstrate microcytic hypochromic erythrocytes with anisocytosis and poikilocytosis, while severe

cases may show pencil cells and marked variation in red-cell morphology.[6]

Iron profile assessment plays a central role in differentiating IDA from other causes of microcytic anemia. Serum ferritin reflects body iron stores and is generally decreased in uncomplicated iron deficiency, whereas serum iron and transferrin saturation are reduced and total iron-binding capacity is usually increased.[7] However, ferritin is an acute-phase reactant and may remain normal or elevated in patients with coexisting infection, inflammation, liver disease, or malignancy, potentially complicating the interpretation of iron status.[8]

Thalassemia represents another important cause of microcytic hypochromic anemia, particularly in regions where hemoglobinopathies are prevalent. Thalassemias are inherited disorders characterized by reduced or absent synthesis of one or more globin chains, resulting in ineffective erythropoiesis and varying degrees of anemia.[9] The peripheral blood smear may demonstrate marked microcytosis, hypochromia, target cells, anisopoikilocytosis, and nucleated red blood cells depending on disease severity. In contrast to IDA, microcytosis in thalassemia trait may be disproportionately severe relative to the degree of anemia, with a relatively preserved or increased red blood cell count.[10]

Hemoglobin electrophoresis and high-performance liquid chromatography (HPLC) are important investigations for the detection and characterization of hemoglobin disorders. In β -thalassemia trait, an increased HbA₂ fraction is a characteristic finding, whereas other abnormal hemoglobin fractions may indicate structural hemoglobin variants or different hemoglobinopathies.[11] Identification of thalassemia trait is particularly important because unnecessary iron supplementation can be avoided and appropriate genetic counseling can be offered.

Routine complete blood count parameters and peripheral smear morphology remain useful initial tools in the evaluation of microcytic anemia. Red-cell indices such as MCV, MCH, mean corpuscular hemoglobin concentration (MCHC), red-cell distribution width (RDW), and RBC count may provide important clues regarding the underlying etiology. Various mathematical indices, including the Mentzer index, have also been proposed to differentiate IDA from thalassemia trait, although confirmatory biochemical and hemoglobin

studies remain necessary.[12] Correlating morphological findings with iron parameters and hemoglobin electrophoresis may therefore provide a systematic approach to the etiological diagnosis of microcytic hypochromic anemia. Such an integrated approach is particularly relevant in populations where nutritional iron deficiency and inherited hemoglobin disorders coexist. The present study was undertaken to evaluate the morphological spectrum of microcytic hypochromic anemia and correlate hematological findings with iron profile and hemoglobin electrophoresis.

MATERIALS AND METHODS

This was a hospital-based cross-sectional observational study conducted in the Department of Pathology at a tertiary care teaching hospital. Patients whose complete blood count and peripheral blood smear demonstrated microcytic hypochromic anemia during the study period were screened for eligibility. A total of 130 patients with microcytic hypochromic anemia fulfilling the eligibility criteria were included in the study. Patients were enrolled consecutively during the study period.

Inclusion Criteria

Patients fulfilling the following criteria were included:

1. Patients of either sex presenting with anemia.
2. Hemoglobin concentration below the reference range appropriate for age and sex.
3. Presence of microcytosis and hypochromia on automated hematological analysis and/or peripheral blood smear examination.
4. Patients for whom adequate clinical and laboratory information was available.
5. Patients providing informed consent where applicable.

Exclusion Criteria

The following patients were excluded:

1. Patients who had received a blood transfusion within the preceding three months.
2. Patients receiving iron therapy for a prolonged period before evaluation.
3. Patients with known hematological malignancies.
4. Patients with severe acute blood loss.
5. Patients with incomplete hematological, iron-profile, or relevant confirmatory investigation data.

6. Samples that were clotted, hemolyzed, inadequate, or unsuitable for analysis.

Clinical Assessment

Relevant demographic and clinical information was recorded using a structured study proforma. Data included age, sex, presenting symptoms, dietary history, history of chronic blood loss, menstrual history in females, gastrointestinal symptoms, previous transfusions, family history of anemia or hemoglobinopathy, previous iron supplementation, and relevant comorbidities.

Sample Collection

Venous blood was collected under aseptic precautions. Blood collected in an EDTA tube was used for complete blood count, peripheral smear examination, and hemoglobin electrophoresis/HPLC, as applicable. Serum separated from blood collected in an appropriate plain tube was used for iron-profile investigations.

Complete Blood Count

Complete blood count was performed using an automated hematology analyzer. The following parameters were recorded:

- Hemoglobin (Hb)
- Red blood cell count (RBC count)
- Hematocrit/packed cell volume (PCV)
- Mean corpuscular volume (MCV)
- Mean corpuscular hemoglobin (MCH)
- Mean corpuscular hemoglobin concentration (MCHC)
- Red-cell distribution width (RDW)
- Total leukocyte count
- Platelet count

Anemia was graded according to appropriate standard hemoglobin thresholds.

Peripheral Blood Smear Examination

Peripheral blood smears were prepared from fresh EDTA-anticoagulated blood and stained using a Romanowsky stain. Smears were examined systematically under light microscopy.

Red-cell morphology was assessed for:

- Microcytosis
- Hypochromia
- Anisocytosis
- Poikilocytosis
- Target cells
- Pencil/elliptical cells
- Tear-drop cells
- Polychromasia
- Basophilic stippling
- Nucleated red blood cells

- Other abnormal morphological findings

The predominant morphological pattern and severity of microcytosis and hypochromia were documented.

Iron Profile

Iron status was evaluated using the following biochemical parameters:

- Serum ferritin
- Serum iron
- Total iron-binding capacity (TIBC)
- Transferrin saturation

Transferrin saturation was calculated from serum iron and TIBC. Iron deficiency was diagnosed on the basis of reduced serum ferritin and/or an iron-profile pattern consistent with depleted iron stores, interpreted in the clinical context.

Hemoglobin Electrophoresis/HPLC

Hemoglobin analysis was performed in patients according to the study protocol using hemoglobin electrophoresis and/or high-performance liquid chromatography (HPLC).

The following hemoglobin fractions were assessed where applicable:

- HbA
- HbA₂
- HbF
- HbE
- HbS
- Other abnormal hemoglobin fractions

An increased HbA₂ level, together with appropriate hematological findings, was considered supportive of β -thalassemia trait. Other abnormal fractions were interpreted according to established diagnostic criteria.

Mentzer Index

The Mentzer index was calculated from routine hematological parameters as:

Mentzer index = MCV (fL) / RBC count (million/ μ L)

A value **<13** was considered suggestive of thalassemia trait, whereas a value **>13** favored iron deficiency anemia. The diagnostic performance of the Mentzer index was assessed against iron-profile and hemoglobin-analysis findings.

Etiological Classification

Based on clinical history, peripheral blood morphology, iron profile, and hemoglobin analysis, patients were categorized into the following etiological groups:

- Iron deficiency anemia
- β -thalassemia trait
- Other hemoglobinopathies

- Anemia of chronic disease/inflammation
- Combined iron deficiency and hemoglobinopathy
- Other/undetermined causes

Correlation of Morphology with Iron Profile

The relationship between morphological features and iron parameters was evaluated. The severity of microcytosis and hypochromia, MCV, MCH, RDW, and other RBC parameters were correlated with serum ferritin, serum iron, TIBC, and transferrin saturation.

Correlation with Hemoglobin Electrophoresis/HPLC

RBC indices and peripheral smear findings were compared between patients with normal and abnormal hemoglobin analysis. Particular emphasis was placed on RBC count, MCV, MCH, RDW, target cells, and Mentzer index in differentiating IDA from β -thalassemia trait.

Outcome Measures

The **primary outcome** was the etiological and morphological spectrum of microcytic hypochromic anemia based on peripheral blood smear, iron profile, and hemoglobin analysis. Secondary outcomes included the correlation of RBC indices and morphological features with iron status, the relationship between hematological parameters and HbA₂ levels, and the diagnostic utility of the Mentzer index for differentiating IDA from β -thalassemia trait.

Statistical Analysis

Data were entered into a spreadsheet and analyzed using SPSS. 17 statistical software. Continuous variables were expressed as mean $\pm$ standard deviation or median with

interquartile range according to their distribution, while categorical variables were presented as frequencies and percentages.

Normality was assessed using the Shapiro–Wilk test. Continuous variables between two groups were compared using the independent-samples t-test or Mann–Whitney U test, as appropriate. ANOVA or the Kruskal–Wallis test was used for comparisons involving more than two groups. Categorical variables were compared using the Chi-square test or Fisher's exact test.

The relationship between hematological parameters and iron-profile variables was evaluated using Pearson or Spearman correlation coefficients. Diagnostic performance of the Mentzer index and selected RBC indices for differentiating IDA from β -thalassemia trait was evaluated using receiver operating characteristic (ROC) curve analysis. Sensitivity, specificity, positive predictive value, negative predictive value, accuracy, and area under the curve (AUC) were calculated where appropriate. A p-value <0.05 was considered statistically significant.

RESULTS

A total of 130 patients with microcytic hypochromic anemia were included in the study. The mean age was 34.8 ± 15.2 years, with the largest proportion belonging to the 21–40-year age group (43.1%). Females constituted 76 (58.5%) patients, giving a female predominance. The mean hemoglobin concentration was 8.7 ± 1.8 g/dL, mean MCV was 67.8 ± 7.9 fL, and mean MCH was 21.1 ± 3.2 pg. The mean RDW was $17.6 \pm 3.4\%$, reflecting considerable variation in red-cell size (Table 1).

Table 1. Demographic and hematological characteristics of the study population (N=130)

Variable	Value
Age (years), mean $\pm$ SD	34.8 ± 15.2
≤ 20 years, n (%)	27 (20.8)
21–40 years, n (%)	56 (43.1)
41–60 years, n (%)	34 (26.2)
>60 years, n (%)	13 (10.0)
Male, n (%)	54 (41.5)
Female, n (%)	76 (58.5)
Hemoglobin (g/dL), mean $\pm$ SD	8.7 ± 1.8
RBC count (million/ μ L), mean $\pm$ SD	4.62 ± 0.91
Hematocrit (%), mean $\pm$ SD	29.4 ± 5.8
MCV (fL), mean $\pm$ SD	67.8 ± 7.9
MCH (pg), mean $\pm$ SD	21.1 ± 3.2
MCHC (g/dL), mean $\pm$ SD	29.8 ± 2.4
RDW (%), mean $\pm$ SD	17.6 ± 3.4

Peripheral blood smear examination demonstrated microcytosis and hypochromia in all patients as defined by the study inclusion criteria. Anisocytosis was observed in 106 (81.5%) patients and poikilocytosis in 91 (70.0%). Pencil cells were present in 66 (50.8%) patients and were particularly frequent

among patients with iron deficiency. Target cells were identified in 35 (26.9%), while basophilic stippling was present in 13 (10.0%) patients. Target cells and basophilic stippling were more frequently associated with hemoglobin disorders (Table 2).

Table 2. Peripheral blood smear morphological findings (N=130)

Morphological finding	n (%)
Microcytosis	130 (100.0)
Hypochromia	130 (100.0)
Anisocytosis	106 (81.5)
Poikilocytosis	91 (70.0)
Pencil/elliptical cells	66 (50.8)
Target cells	35 (26.9)
Tear-drop cells	18 (13.8)
Basophilic stippling	13 (10.0)
Polychromasia	11 (8.5)
Nucleated RBCs	5 (3.8)

Based on the combined assessment of peripheral smear morphology, iron profile, clinical findings, and hemoglobin analysis, iron deficiency anemia (IDA) was the most common diagnosis, accounting for 78 (60.0%) patients. β -thalassemia trait was identified in 24 (18.5%), anemia of chronic

disease/inflammation in 12 (9.2%), and combined IDA with β -thalassemia trait in 8 (6.2%). Other hemoglobinopathies were identified in 5 (3.8%) patients, while the etiology remained undetermined in 3 (2.3%) (Table 3).

Table 3. Etiological distribution of microcytic hypochromic anemia (N=130)

Etiology	n (%)
Iron deficiency anemia	78 (60.0)
β -thalassemia trait	24 (18.5)
Anemia of chronic disease/inflammation	12 (9.2)
Combined IDA + β -thalassemia trait	8 (6.2)
Other hemoglobinopathies	5 (3.8)
Undetermined/other	3 (2.3)
Total	130 (100.0)

A detailed comparison was performed between patients with isolated IDA and β -thalassemia trait. Patients with β -thalassemia trait had a significantly higher RBC count than those with IDA (5.61 ± 0.63 vs 4.18 ± 0.67 million/ μ L; $p < 0.001$), despite having a significantly lower MCV (62.4 ± 5.6 vs 68.9 ± 6.7 fL; $p < 0.001$). RDW was significantly higher in IDA ($19.1 \pm$

3.1% vs $15.0 \pm 1.8\%$; $p < 0.001$). Serum ferritin and transferrin saturation were markedly lower among patients with IDA, while HbA₂ was significantly elevated among those with β -thalassemia trait. Pencil cells were more common in IDA, whereas target cells and basophilic stippling were more frequent in β -thalassemia trait (Table 4).

Table 4. Comparison of hematological, morphological, and biochemical parameters between IDA and β -thalassemia trait

Parameter	IDA (n=78)	β -thalassemia trait (n=24)	P-value
Hemoglobin (g/dL)	8.4 ± 1.6	10.1 ± 1.3	<0.001
RBC count (million/ μ L)	4.18 ± 0.67	5.61 ± 0.63	<0.001
MCV (fL)	68.9 ± 6.7	62.4 ± 5.6	<0.001
MCH (pg)	20.5 ± 2.8	19.4 ± 2.3	0.07

RDW (%)	19.1 ± 3.1	15.0 ± 1.8	<0.001
Serum ferritin (ng/mL), median (IQR)	8.2 (4.5–13.6)	58.4 (36.2–89.7)	<0.001
Serum iron (µg/dL), mean ± SD	31.8 ± 13.6	82.6 ± 24.7	<0.001
TIBC (µg/dL), mean ± SD	418.5 ± 62.4	311.6 ± 51.8	<0.001
Transferrin saturation (%), mean ± SD	7.8 ± 3.9	26.7 ± 8.4	<0.001
HbA ₂ (%), mean ± SD	2.5 ± 0.4	5.2 ± 0.8	<0.001
Pencil cells, n (%)	53 (67.9)	5 (20.8)	<0.001
Target cells, n (%)	10 (12.8)	19 (79.2)	<0.001
Basophilic stippling, n (%)	3 (3.8)	8 (33.3)	<0.001
Mentzer index, mean ± SD	16.8 ± 3.1	11.2 ± 1.7	<0.001

Correlation analysis among patients with IDA demonstrated significant relationships between red-cell indices and iron status. Serum ferritin showed a positive correlation with hemoglobin ($r=0.48$; $p<0.001$), MCV ($r=0.43$; $p<0.001$), and MCH ($r=0.39$; $p<0.001$), whereas RDW showed a significant negative correlation with

ferritin ($r=-0.46$; $p<0.001$). Transferrin saturation also correlated positively with hemoglobin and MCV. These findings indicated progressively greater microcytosis, hypochromia, and anisocytosis with increasing severity of iron deficiency (Table 5).

Table 5. Correlation of hematological indices with iron-profile parameters among patients with IDA (n=78)

Hematological parameter	Serum ferritin r (p-value)	Serum iron r (p-value)	Transferrin saturation r (p-value)
Hemoglobin	0.48 (<0.001)	0.41 (<0.001)	0.45 (<0.001)
MCV	0.43 (<0.001)	0.36 (0.001)	0.40 (<0.001)
MCH	0.39 (<0.001)	0.34 (0.002)	0.37 (0.001)
RDW	-0.46 (<0.001)	-0.32 (0.004)	-0.38 (0.001)
RBC count	0.18 (0.12)	0.14 (0.22)	0.16 (0.16)

The diagnostic utility of routine hematological indices for differentiating IDA from β -thalassemia trait was further evaluated. A Mentzer index <13 demonstrated a sensitivity of 87.5% and specificity of 89.7% for identifying β -thalassemia trait, with an overall diagnostic accuracy of 89.2%. ROC analysis

demonstrated an AUC of 0.91 (95% CI: 0.84–0.97; $p<0.001$). RBC count >5.0 million/ μ L and RDW $\leq 15\%$ also showed useful discriminatory ability, although their overall diagnostic performance was lower than that of the Mentzer index (Table 6).

Table 6. Diagnostic performance of hematological indices for identifying β -thalassemia trait

Parameter	Mentzer index <13	RBC count >5.0 million/ μ L	RDW $\leq 15\%$
Sensitivity (%)	87.5	83.3	70.8
Specificity (%)	89.7	84.6	82.1
PPV (%)	72.4	62.5	54.8
NPV (%)	95.9	94.3	90.1
Accuracy (%)	89.2	84.3	79.4
AUC	0.91	0.87	0.81
95% CI	0.84–0.97	0.79–0.94	0.71–0.90
p-value	<0.001	<0.001	<0.001

Overall, IDA constituted the predominant cause of microcytic hypochromic anemia, followed by β -thalassemia trait. Peripheral smear morphology showed distinct patterns, with pencil cells and greater anisocytosis being associated predominantly with IDA, whereas

target cells and basophilic stippling were more frequently observed in β -thalassemia trait. Iron parameters demonstrated significant correlations with the severity of hematological abnormalities. The combination of a relatively high RBC count, marked microcytosis, lower

RDW, target cells, and a Mentzer index <13 was strongly suggestive of β -thalassemia trait; however, iron profile and hemoglobin analysis remained essential for definitive etiological classification.

DISCUSSION

The present study evaluated the morphological spectrum of microcytic hypochromic anemia and its correlation with iron profile and hemoglobin electrophoresis/HPLC in 130 patients. Iron deficiency anemia (IDA) was the most common etiology, accounting for 60.0% of cases, followed by β -thalassemia trait in 18.5%, anemia of chronic disease/inflammation in 9.2%, and combined IDA with β -thalassemia trait in 6.2%. The study also demonstrated significant differences in red-cell indices, peripheral smear findings, iron parameters, and HbA₂ levels between IDA and β -thalassemia trait.

The mean age of the study population was 34.8 ± 15.2 years, with a predominance of patients in the 21–40-year age group. Females constituted 58.5% of the cohort. The higher frequency of microcytic anemia among younger and middle-aged women can largely be attributed to increased iron requirements, menstrual blood loss, pregnancy-related iron depletion, and nutritional factors. Similar demographic patterns have been observed in studies evaluating iron deficiency and microcytic anemia in developing countries.[13] In the present study, the mean hemoglobin concentration was 8.7 ± 1.8 g/dL, while the mean MCV and MCH were 67.8 ± 7.9 fL and 21.1 ± 3.2 pg, respectively. These findings reflect the characteristic reduction in red-cell size and hemoglobin content seen in disorders of impaired hemoglobin synthesis. IDA and thalassemia are recognized as the two most common causes of microcytic hypochromic anemia and may overlap considerably in routine hematological parameters.[14]

Peripheral blood smear examination revealed anisocytosis in 81.5% and poikilocytosis in 70.0% of cases. Pencil cells were present in 50.8%, whereas target cells were observed in 26.9%. Pencil cells were significantly more frequent in IDA, while target cells and basophilic stippling were markedly more common in β -thalassemia trait. These morphological differences are in agreement with established hematological patterns, where marked anisocytosis and elliptocytic/pencil forms favor iron deficiency, while prominent

target cells and basophilic stippling suggest a hemoglobin synthesis disorder.[15]

IDA accounted for 60.0% of all cases, making it the dominant cause of microcytic hypochromic anemia in the present study. This finding is consistent with the global and Indian burden of iron deficiency, which remains the most common nutritional deficiency and a major cause of anemia.[16] Iron deficiency develops through progressive depletion of body iron stores, followed by iron-restricted erythropoiesis and ultimately reduced hemoglobin synthesis, resulting in progressive microcytosis and hypochromia.[17]

The biochemical profile of patients with IDA was characteristic, with a median serum ferritin of 8.2 ng/mL, mean serum iron of 31.8 μ g/dL, increased TIBC, and reduced transferrin saturation. In contrast, patients with β -thalassemia trait had significantly higher ferritin and serum iron concentrations with relatively preserved transferrin saturation. Serum ferritin is considered one of the most useful indicators of body iron stores, although its interpretation may be complicated by inflammation because ferritin behaves as an acute-phase reactant.[18]

A major observation of the present study was the significant difference in RBC count between IDA and β -thalassemia trait. Patients with β -thalassemia trait had a significantly higher RBC count than patients with IDA (5.61 ± 0.63 vs 4.18 ± 0.67 million/ μ L; $p < 0.001$), despite having a lower MCV. This pattern is well recognized in thalassemia trait, where erythrocyte production remains relatively preserved despite marked microcytosis.[19] In contrast, iron deficiency is generally associated with a lower RBC count because inadequate iron availability limits erythropoiesis.

RDW was significantly higher in IDA than in β -thalassemia trait ($19.1 \pm 3.1\%$ vs $15.0 \pm 1.8\%$; $p < 0.001$). Increased RDW in IDA reflects heterogeneous red-cell populations resulting from progressive iron depletion and variable degrees of microcytosis. In thalassemia trait, red cells tend to be uniformly microcytic, resulting in relatively lower RDW values. Similar observations have been reported in previous studies comparing red-cell indices in IDA and β -thalassemia trait.[20]

Hemoglobin analysis played an important role in confirming β -thalassemia trait. Mean HbA₂ was significantly elevated in patients with β -thalassemia trait ($5.2 \pm 0.8\%$) compared with those with IDA ($2.5 \pm 0.4\%$; $p < 0.001$). Elevated HbA₂ remains a key laboratory feature

of β -thalassemia trait and forms an important component of its diagnosis when interpreted together with CBC findings and iron status.[21] The presence of combined IDA and β -thalassemia trait in 6.2% of patients is clinically important because coexisting iron deficiency may modify hematological indices and sometimes reduce HbA₂ values, thereby complicating diagnosis.

The present study also demonstrated significant correlations between iron parameters and hematological indices among patients with IDA. Serum ferritin showed positive correlations with hemoglobin, MCV, and MCH, whereas RDW demonstrated a negative correlation with ferritin. These findings indicate that worsening iron depletion is associated with greater anemia, increasing microcytosis and hypochromia, and greater variation in red-cell size. Similar relationships between ferritin, MCV, MCH, and RDW have been described in previous studies of iron deficiency.[02]

The Mentzer index showed good diagnostic performance for differentiating β -thalassemia trait from IDA. A value <13 demonstrated a sensitivity of 87.5%, specificity of 89.7%, and an AUC of 0.91. The Mentzer index is widely used as a simple screening tool and is based on the relationship between MCV and RBC count. Previous studies have also demonstrated its usefulness in distinguishing thalassemia trait from iron deficiency, particularly in resource-limited settings.[21]

However, no single hematological index is sufficiently accurate to establish the diagnosis in all patients. The presence of coexisting iron deficiency, chronic inflammation, mixed hemoglobin disorders, or other causes of microcytosis may alter routine indices and reduce diagnostic accuracy. Therefore, screening indices should primarily be used to identify patients who require confirmatory investigations rather than as substitutes for iron profile and hemoglobin analysis.[21]

From a practical perspective, the present findings support a stepwise diagnostic approach to microcytic hypochromic anemia. CBC parameters and peripheral smear examination can provide important initial clues regarding the underlying cause. A low ferritin level with increased RDW and pencil cells strongly favors IDA, whereas a relatively high RBC count, marked microcytosis, target cells, low Mentzer index, and elevated HbA₂ suggest β -thalassemia trait. Integration of morphology, red-cell indices, iron studies, and hemoglobin

analysis can therefore improve diagnostic accuracy and reduce inappropriate iron therapy.

CONCLUSION

Iron deficiency anemia was the most common cause of microcytic hypochromic anemia, followed by β -thalassemia trait. RBC count, RDW, peripheral smear morphology, iron profile, and HbA₂ levels showed significant differences between these two conditions. A combined approach using morphology, hematological indices, iron studies, and hemoglobin analysis provides better etiological differentiation than any single parameter alone.

Limitations

The study was limited by its single-centre design and relatively modest sample size of 130 patients. Molecular testing for α - and β -thalassemia mutations was not performed, which may have limited characterization of certain hemoglobin disorders. In addition, inflammatory markers that could influence ferritin interpretation were not uniformly assessed in all patients.

REFERENCES

1. Boopathy T, Subathra J, Sangeetha N, Karthika VV. Haematological profile in microcytic hypochromic anaemia in children. *IOSR J Dent Med Sci*. 2017;16(1):100-4.
2. Borgna-Pignatti C, Galanello R. Thalassemias and related disorders: quantitative disorders of hemoglobin synthesis. In: Greer JP, Arber DA, Glader B, List AF, Means RTJ, Paraskevas F, et al., editors. *Wintrobe's Clinical Hematology*. 13th ed. Lippincott Williams and Wilkins; 2014. p. 862-913.
3. Wharton BA. Iron deficiency in children: detection and prevention. *Br J Haematol*. 1999;106(2):270-80.
4. Irwin JJ, Kirchner JT. Anemia in children. *Am Fam Physician*. 2001;64(8):1379-86.
5. Baral KP, Onta SR. Prevalence of anemia amongst adolescents in Nepal: a community based study in rural and urban areas of Morang District. *Nepal Med Coll J*. 2009;11(3):179-82.
6. World Health Organization. The global prevalence of anaemia in 2011. Geneva: World Health Organization; 2015.
7. Vehapoglu A, Ozgurhan G, Demir AD, Uzuner S, Nursoy MA, Turkmen S, et al. Hematological indices for differential diagnosis of beta thalassemia trait and

- iron deficiency anemia. *Anemia*. 2014;2014:1-7.
8. Mentzer WC Jr. Differentiation of iron deficiency from thalassemia trait. *Lancet*. 1973;1:882.
9. Shine I, Lal S. A strategy to detect beta-thalassaemia minor. *Lancet*. 1977;1:692-4.
10. England JM, Fraser PM. Differentiation of iron deficiency from thalassaemia trait by routine blood-count. *Lancet*. 1973;1:449-52.
11. Srivastava PC. Differentiation of thalassemia minor from iron deficiency. *Lancet*. 1973;2:155-6.
12. Bessman JD, Feinstein DI. Quantitative anisocytosis as a discriminant between iron deficiency and thalassemia minor. *Blood*. 1979;53(2):288-93.
13. Sirdah M, Tarazi I, Najjar EAL, Haddad RAL. Evaluation of the diagnostic reliability of different RBC indices and formulas in the differentiation of the β -thalassaemia minor from iron deficiency in Palestinian population. *Int J Lab Hematol*. 2008;30(4):324-30.
14. Ntaios G, Chatzinikolaou A, Saouli Z, Girtovitis F, Tsapanidou M, Kaiafa G, et al. Discrimination indices as screening tests for β -thalassemic trait. *Ann Hematol*. 2007;86:487-91.
15. Nalbantolu B, Güzel S, Büyükyalçın V, Donma MM, Güzel EÇ, Nalbantolu A, et al. Indices used in differentiation of thalassemia trait from iron deficiency anemia in pediatric population: are they reliable? *Pediatr Hematol Oncol*. 2012;29(5):472-8.
16. Khatiwada S, Lamsal M, Gelal B, Gautam S, Nepal AK, Brodie D, et al. Anemia, iron deficiency and iodine deficiency among Nepalese school children. *Indian J Pediatr*. 2015;83(7):617-21.
17. Weippl G, Pantlitschko M, Bauer P, Lund S. Serumeisen-Normalwerte und statistische Verteilung der Einzelwerte bei Mann und Frau. *Blut*. 1973;27(4):261-9.
18. Shoushtari AN, Parameswaran R. Benign hematology. In: *Pocket Oncology (Pocket Notebook Series)*. 1st ed.
19. Ghosh A, Ghartimagar D, Thapa S, Sathian B, De A. Microcytic hypochromic anemia in pediatric age group: a hospital based study in Nepal. *Am J Public Health Res*. 2015;3(4A):57-61.
20. Looker AC, Dallman PR, Carroll MD, Gunter EW, Johnson CL. Prevalence of iron deficiency in the United States. *J Am Med Assoc*. 1997;277(12):973-6.
21. Viswanath D, Hegde R, Murthy V, Nagashree S, Shah R. Red cell distribution width in the diagnosis of iron deficiency anemia. *Indian J Pediatr*. 2001;68:1117-9.