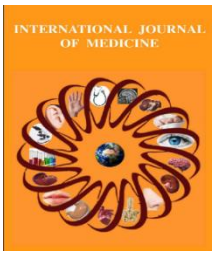
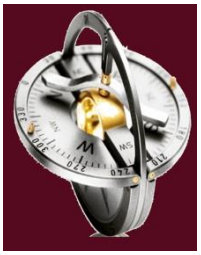


International Journal of Medicine
ISSN (P): 1468-3814; ISSN (e): 2667-7008

https://www.theinternationalmedicine.com



Volume: 6(1) 2024

Original Research

Cross-Sectional Study of Red Blood Cell Morphology in Iron Deficiency Anemia vs. Thalassemia.

Dr. Narendra Ramrao Patil[1*], Dr.Sushilkumar Bhagwanrao Dodke[2], Dr.Khan Sanober Muzammil Mir[3],
[1]*Professor, Department of Pathology, Parbhani Medical College, Parbhani, Maharashtra, INDIA.*
[2]*Assistant Professor, Department of Pathology, Parbhani Medical College, Parbhani, Maharashtra, INDIA.*
[3]*Assistant Professor, Department of Pathology, Parbhani Medical College, Parbhani, Maharashtra, INDIA.*

Received: 08 Sep 2024 / Accepted: 30 Nov 2024

Abstract

Background: Iron deficiency anemia (IDA) and thalassemia are among the most common causes of microcytic hypochromic anemia. Although both conditions share similar hematological presentations, their pathophysiology, treatment, and prognosis differ significantly. Peripheral blood smear examination remains an important and economical diagnostic tool for differentiating these disorders, particularly in resource-limited settings. *Aim:* To study and compare red blood cell morphology in patients with iron deficiency anemia and thalassemia. *Objectives:* To evaluate the peripheral blood smear morphology of red blood cells in patients with iron deficiency anemia and thalassemia. To compare the morphological characteristics of red blood cells between iron deficiency anemia and thalassemia patients. *Materials and Methods:* This hospital-based cross-sectional observational study was conducted in the Department of Pathology of a tertiary care teaching hospital over a period of 18 months. A total of 130 patients diagnosed with iron deficiency anemia or thalassemia were included in the study. Venous blood samples were collected in EDTA vacutainers and analyzed using automated hematology analyzers. Peripheral blood smears were prepared and stained using Leishman stain for morphological evaluation. Hematological parameters including hemoglobin, MCV, MCH, MCHC, RDW, RBC count, and serum ferritin were analyzed. Morphological features such as microcytosis, hypochromia, anisocytosis, target cells, pencil cells, basophilic stippling, nucleated RBCs, and polychromasia were compared between the two groups. Statistical analysis was performed using SPSS software, and p-value <0.05 was considered statistically significant. *Results:* The mean hemoglobin level was slightly lower in IDA patients compared to thalassemia patients. MCV was significantly lower in thalassemia, whereas RDW was significantly higher in IDA patients ($p<0.001$). RBC count was significantly lower in IDA and relatively preserved in thalassemia patients ($p<0.001$). Peripheral smear examination revealed that anisocytosis and pencil cells were significantly more common in IDA, while target cells, basophilic stippling, nucleated RBCs, and polychromasia were significantly associated with thalassemia ($p<0.001$). Marked hypochromia with low RBC count and high RDW pattern were characteristic of IDA, whereas microcytosis disproportionate to anemia and target cell predominance favored thalassemia. *Conclusion:* Peripheral smear morphology combined with routine hematological parameters provides an effective and economical approach for differentiating iron deficiency anemia from thalassemia. Features such as high RDW, anisopoikilocytosis, and pencil cells favored IDA, whereas target cells, basophilic stippling, nucleated RBCs, and relatively preserved RBC count favored thalassemia. Careful morphological evaluation remains valuable in routine hematological practice.

Keywords: *Iron Deficiency Anemia. Thalassemia. Red Blood Cell Morphology.*

Introduction

Iron deficiency anemia (IDA) and thalassemia are among the most common causes of microcytic hypochromic anemia worldwide and represent significant public health problems, particularly in developing countries. Both conditions frequently present with reduced hemoglobin levels and altered red blood cell (RBC) indices; however, their underlying etiologies, pathophysiology, treatment approaches, and prognostic implications differ considerably. Iron deficiency anemia occurs due to inadequate iron availability for hemoglobin synthesis, commonly resulting from nutritional deficiency, chronic blood loss, malabsorption, or increased physiological demands. In contrast, thalassemia is an inherited hemoglobinopathy characterized by defective globin chain synthesis leading to ineffective erythropoiesis and hemolysis. Accurate differentiation between these two disorders is essential to avoid unnecessary iron therapy in thalassemia patients and to ensure timely management of iron deficiency anemia.[1].

Red blood cell morphology on peripheral blood smear examination plays a crucial role in the preliminary diagnosis and differentiation of hematological disorders. Morphological evaluation provides important information regarding the size, shape, color, and inclusions within erythrocytes. In iron deficiency anemia, the peripheral smear commonly demonstrates microcytic hypochromic red cells with marked anisopoikilocytosis, pencil cells, and occasional target cells. On the other hand, thalassemia often shows microcytosis disproportionate to the degree of anemia, numerous target cells, basophilic stippling, nucleated red blood cells, and relatively preserved RBC counts. Careful examination of RBC morphology, along with hematological indices, may therefore assist in differentiating IDA from thalassemia in resource-limited settings where advanced diagnostic modalities may not always be readily available.[2].

Address for Correspondence Dr. Narendra Ramrao Patil, Professor, Department of Pathology, Parbhani Medical College, Parbhani, Maharashtra, INDIA.
.

DOI: 10.61336/im/24-1-22

Despite advances in automated hematology analyzers and molecular diagnostic techniques, peripheral smear examination remains a simple, inexpensive, and widely accessible diagnostic tool. Several RBC indices such as mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), red cell distribution width (RDW), and Mentzer index are frequently utilized to distinguish between IDA and thalassemia; however, overlap in values may occur. Morphological assessment can complement these indices and improve diagnostic accuracy. Understanding the characteristic morphological differences between IDA and thalassemia may aid clinicians and pathologists in early diagnosis, appropriate referral, and management planning.[3]

AIM
To study and compare red blood cell morphology in patients with iron deficiency anemia and thalassemia.

OBJECTIVES
1. To evaluate the peripheral blood smear morphology of red blood cells in patients with iron deficiency anemia and thalassemia.
2. To compare the morphological characteristics of red blood cells between iron deficiency anemia and thalassemia patients.

Materials and Methods

Source of Data
The data for the present study were collected from patients attending the Department of Pathology and associated clinical departments of the tertiary care teaching hospital. Hematological investigations, peripheral blood smear findings, and relevant clinical details of patients diagnosed with iron deficiency anemia or thalassemia were included in the study.

Study Design
The present study was conducted as a hospital-based cross-sectional observational study.

Study Location
The study was carried out in the Department of Pathology at a tertiary care teaching hospital with collaboration from the Departments of Medicine and Pediatrics.

Study Duration
The study was conducted over a period of 18 months from the date of approval by the Institutional Ethics Committee.

Sample Size
A total of 130 patients were included in the study. The study population consisted of patients diagnosed with iron deficiency anemia and thalassemia based on clinical evaluation and laboratory investigations.

Inclusion Criteria
1. Patients of all age groups and both genders diagnosed with iron deficiency anemia.
2. Patients diagnosed with thalassemia based on hematological and electrophoretic findings.
3. Patients willing to participate in the study and provide informed consent.
4. Patients with complete hematological investigation records and peripheral smear examination.

Exclusion Criteria
1. Patients with dimorphic anemia.
2. Patients with anemia due to chronic diseases, renal disorders, liver disorders, or malignancy.
3. Patients who had received blood transfusion within the previous three months.
4. Patients on iron therapy or hematinic supplementation before sample collection.
5. Inadequate or hemolyzed blood samples.

Procedure and Methodology
After obtaining approval from the Institutional Ethics Committee, patients fulfilling the inclusion criteria were enrolled in the study. Written informed consent was obtained from all participants or guardians in the case of pediatric patients. Detailed clinical history including age, gender, presenting complaints, dietary history, family history, and previous treatment history was recorded in a predesigned proforma.

Approximately 2-3 mL of venous blood was collected aseptically in EDTA vacutainers for hematological analysis. Complete blood count (CBC) was performed using an automated hematology analyzer to obtain parameters including hemoglobin concentration, RBC count, hematocrit, MCV, MCH, MCHC, and RDW.

Peripheral blood smears were prepared immediately after sample collection and stained using Leishman stain. Smears were examined under light microscopy by experienced pathologists. Red blood cell morphology was assessed systematically for size, shape, color, and presence of abnormal forms such as microcytes, hypochromia, anisocytosis, poikilocytosis, target cells, pencil cells, tear drop cells, basophilic stippling, and nucleated red blood cells.

Iron deficiency anemia was diagnosed based on hematological findings and supportive biochemical investigations such as serum ferritin, serum iron, and total iron-binding capacity wherever available. Thalassemia cases were identified based on hemoglobin electrophoresis or high-performance liquid chromatography (HPLC) findings along with hematological parameters.
The morphological findings in peripheral smears of iron deficiency anemia patients were compared with those observed in thalassemia patients to identify distinguishing features.

Sample Processing
Blood samples were processed immediately after collection to avoid morphological distortion of red blood cells. Automated hematological analysis was performed according to standard laboratory protocols. Peripheral smears were air-dried, stained with Leishman stain, and examined under oil immersion microscopy for detailed morphological assessment.

Quality control procedures for staining, microscopy, and analyzer calibration were maintained throughout the study period to ensure reliability and reproducibility of results.

Statistical Methods
The collected data were entered into Microsoft Excel and analyzed using Statistical Package for Social Sciences (SPSS) software version 25.0. Quantitative variables were expressed as mean ± standard deviation, while qualitative variables were presented as frequencies and percentages.
Comparisons between iron deficiency anemia and thalassemia groups were performed using Chi-square test for categorical variables and Student's t-test for continuous variables. A p-value of less than 0.05 was considered statistically significant.

Data Collection

Data collection was carried out using a structured case record form. Demographic details, clinical findings, laboratory parameters, and peripheral smear morphology findings were systematically documented. All collected data were maintained confidentially and used solely for research purposes.

Results

Table 1: Comparison of Hematological Parameters between Iron Deficiency Anemia and Thalassemia Patients

Parameter	IDA (n=67) Mean ± SD	Thalassemia (n=63) Mean ± SD	Test value	95% CI	p-value
Hemoglobin (g/dL)	8.7 ± 1.1	9.1 ± 1.2	t=1.98	-0.80 to 0.00	0.050
MCV (fL)	64.8 ± 5.9	61.2 ± 4.8	t=3.83	1.74 to 5.46	<0.001*
MCH (pg)	20.1 ± 2.7	19.4 ± 2.5	t=1.53	-0.20 to 1.60	0.127
MCHC (g/dL)	29.8 ± 2.4	31.3 ± 2.1	t=3.80	-2.28 to -0.72	<0.001*
RDW (%)	16.9 ± 2.6	14.1 ± 1.9	t=7.04	2.01 to 3.59	<0.001*
RBC count (million/µL)	3.71 ± 0.62	5.28 ± 0.81	t=12.35	-1.82 to -1.32	<0.001*
Serum ferritin (ng/mL)	9.8 ± 6.2	38.7 ± 14.8	t=14.36	-32.90 to -24.90	<0.001*

Table 1 compares the hematological parameters between patients with iron deficiency anemia (IDA) and thalassemia. The mean hemoglobin level was slightly lower in the IDA group (8.7 ± 1.1 g/dL) compared to the thalassemia group (9.1 ± 1.2 g/dL), with borderline statistical significance (t=1.98, p=0.050). The mean MCV was significantly higher in IDA patients (64.8 ± 5.9 fL) than in thalassemia patients (61.2 ± 4.8 fL), indicating more severe microcytosis in thalassemia (t=3.83, p<0.001). Similarly, MCH values were marginally higher in IDA (20.1 ± 2.7 pg) than in thalassemia (19.4 ± 2.5 pg), although this difference was not statistically significant (p=0.127).

The mean MCHC was significantly lower in the IDA group (29.8 ± 2.4 g/dL) compared to thalassemia patients (31.3 ± 2.1 g/dL), suggesting more pronounced hypochromia in IDA (t=3.80, p<0.001). RDW was markedly elevated in IDA patients (16.9 ± 2.6%) in comparison to thalassemia patients (14.1 ± 1.9%), and this difference was highly significant (t=7.04, p<0.001), reflecting greater variation in red cell size in IDA. The RBC count was significantly lower in IDA patients (3.71 ± 0.62 million/µL) than in thalassemia patients (5.28 ± 0.81 million/µL) (t=12.35, p<0.001), indicating relatively preserved erythropoiesis in thalassemia. Serum ferritin levels were markedly reduced in IDA (9.8 ± 6.2 ng/mL) compared to thalassemia (38.7 ± 14.8 ng/mL), and this difference was highly significant (t=14.36, p<0.001).

Table 2: Peripheral Blood Smear Morphology in Iron Deficiency Anemia and Thalassemia Patients

RBC Morphological Finding	IDA (n=67) n (%)	Thalassemia (n=63) n (%)	Test value	95% CI/OR	p-value
Microcytosis	64 (95.5)	61 (96.8)	χ^2=0.15	OR=0.70; 0.11-4.33	0.699
Hypochromia	62 (92.5)	60 (95.2)	χ^2=0.41	OR=0.62; 0.14-2.71	0.522
Anisocytosis	59 (88.1)	43 (68.3)	χ^2=7.54	OR=3.43; 1.38-8.52	0.006*
Target cells	18 (26.9)	52 (82.5)	χ^2=40.50	OR=0.08; 0.03-0.18	<0.001*
Pencil cells	39 (58.2)	16 (25.4)	χ^2=14.32	OR=4.09; 1.94-8.63	<0.001*
Basophilic stippling	7 (10.4)	34 (54.0)	χ^2=28.48	OR=0.10; 0.04-0.25	<0.001*
Tear drop cells	4 (6.0)	12 (19.0)	χ^2=5.14	OR=0.27; 0.08-0.89	0.023*
Nucleated RBCs	1 (1.5)	11 (17.5)	χ^2=9.88	OR=0.07; 0.01-0.57	0.002*
Polychromasia	3 (4.5)	18 (28.6)	χ^2=13.92	OR=0.12; 0.03-0.42	<0.001*

Table 2 shows the comparison of peripheral blood smear morphology between iron deficiency anemia and thalassemia patients. Microcytosis was observed in the majority of patients in both groups, being present in 95.5% of IDA patients and 96.8% of thalassemia patients, with no statistically significant difference (p=0.699). Similarly, hypochromia was seen in 92.5% of IDA patients and 95.2% of thalassemia patients, which was also not statistically significant (p=0.522). These findings indicate that both conditions commonly present as microcytic hypochromic anemia.

Anisocytosis was significantly more common in IDA patients, being observed in 88.1% compared to 68.3% of thalassemia patients (χ^2=7.54, p=0.006). Target cells were markedly more frequent in thalassemia patients (82.5%) than in IDA patients (26.9%), showing a highly significant association with thalassemia (χ^2=40.50, p<0.001). Pencil cells were predominantly seen in IDA patients, occurring in 58.2% compared to 25.4% in thalassemia patients (χ^2=14.32, p<0.001).

Basophilic stippling was significantly more frequent in thalassemia patients (54.0%) than in IDA patients (10.4%) (χ^2=28.48, p<0.001). Tear drop cells were also more common in thalassemia (19.0%) compared to IDA (6.0%), with statistical significance (p=0.023). Nucleated RBCs and polychromasia were significantly associated with thalassemia, observed in 17.5% and 28.6% of thalassemia patients respectively, compared to only 1.5% and 4.5% in IDA patients (p=0.002 and p<0.001 respectively).

Table 3: Comparative Diagnostic Morphological Characteristics of RBCs between IDA and Thalassemia

Diagnostic Characteristic	IDA (n=67) n (%)	Thalassemia (n=63) n (%)	Test value	95% CI/OR	p-value
Predominant anisopoikilocytosis	57 (85.1)	38 (60.3)	χ^2=10.17	OR=3.77; 1.65-8.61	0.001*
Marked hypochromia with low RBC count	48 (71.6)	17 (27.0)	χ^2=25.88	OR=6.82; 3.17-14.67	<0.001*
Microcytosis disproportionate to anemia	19 (28.4)	51 (81.0)	χ^2=36.20	OR=0.09; 0.04-0.21	<0.001*
Target cell predominance	16 (23.9)	49 (77.8)	χ^2=37.68	OR=0.09; 0.04-0.20	<0.001*
Pencil/elliptocyte predominance	42 (62.7)	13 (20.6)	χ^2=23.67	OR=6.46; 2.92-14.30	<0.001*
Basophilic stippling predominance	6 (9.0)	31 (49.2)	χ^2=25.33	OR=0.10; 0.04-0.26	<0.001*
High RDW pattern	53 (79.1)	22 (34.9)	χ^2=25.92	OR=7.06; 3.20-15.59	<0.001*
Relatively preserved/increased RBC count	14 (20.9)	48 (76.2)	χ^2=39.78	OR=0.08; 0.04-0.19	<0.001*

Table 3 compares the diagnostic morphological characteristics of red blood cells between iron deficiency anemia and thalassemia patients. Predominant anisopoikilocytosis was significantly more common in IDA patients, observed in 85.1% compared to 60.3% of thalassemia patients (χ^2=10.17, p=0.001). Marked hypochromia associated with low RBC count was also significantly more frequent in IDA patients (71.6%) than in thalassemia patients (27.0%) (χ^2=25.88, p<0.001), indicating severe iron deficiency-related impairment in erythropoiesis.

In contrast, microcytosis disproportionate to the degree of anemia was predominantly observed in thalassemia patients, being present in 81.0% compared to only 28.4% of IDA patients (χ^2=36.20, p<0.001). Target cell predominance was another major feature associated with thalassemia, seen in 77.8% of patients versus 23.9% in IDA patients (χ^2=37.68, p<0.001). Similarly, basophilic stippling predominance was significantly higher in thalassemia patients (49.2%) than in IDA patients (9.0%) (χ^2=25.33, p<0.001).

Pencil or elliptocyte predominance was strongly associated with IDA, occurring in 62.7% of IDA patients compared to only 20.6% of

thalassemia patients (χ^2=23.67, p<0.001). High RDW pattern was significantly more common in IDA patients (79.1%) than in thalassemia patients (34.9%), reflecting greater red cell size variation in iron deficiency anemia (χ^2=25.92, p<0.001). Relatively preserved or increased RBC count was predominantly observed in thalassemia patients (76.2%) compared to IDA patients (20.9%) (χ^2=39.78, p<0.001).

Discussion

In the present study, hematological parameters showed clear differences between iron deficiency anemia (IDA) and thalassemia. Hemoglobin was slightly lower in IDA patients (8.7 ± 1.1 g/dL) than in thalassemia patients (9.1 ± 1.2 g/dL), with borderline significance. However, MCV was significantly lower in thalassemia, while RDW was significantly higher in IDA. This supports the concept that thalassemia produces marked microcytosis disproportionate to anemia, whereas IDA produces greater variation in red cell size due to progressive iron depletion. The RBC count was significantly lower in IDA and relatively preserved or increased in thalassemia, which is one of the most useful hematological differences between the two conditions. Ford et al. (2013)[1] emphasized that careful assessment of RBC morphology and indices remains fundamental in differentiating causes of microcytic anemia. Jassim et al. (2016)[2] similarly demonstrated that RBC count was significantly higher in β-thalassemia trait, whereas RDW was markedly elevated in iron deficiency anemia. Jameel et al. (2017)[7] also reported that RBC count was higher in beta-thalassemia trait, while RDW was more useful in identifying IDA. Ayyıldız et al. (2020)[8] further showed that red cell parameters including MCV, MCH, RDW, and RBC count could effectively discriminate between IDA and β-thalassemia using machine learning-based models.

In the present study, serum ferritin was markedly lower in IDA patients (9.8 ± 6.2 ng/mL) compared with thalassemia patients (38.7 ± 14.8 ng/mL), and this difference was highly significant. This confirms that depleted iron stores remain a major diagnostic feature of IDA, whereas thalassemia is primarily a disorder of globin chain synthesis rather than iron deficiency. Sun et al. (2023)[5] emphasized that differentiation between IDA and thalassemia trait-induced anemia requires combined evaluation of iron studies and hematological indices. Düzenli Kar et al. (2021)[12] reported that reticulocyte hemoglobin equivalent and serum iron parameters were useful markers in distinguishing iron deficiency from β-thalassemia trait in pediatric patients. Lei et al. (2019)[9] also highlighted that alterations in red blood cell lifespan and erythrocyte turnover may aid in differentiating IDA from thalassemia.

Peripheral smear morphology in the present study showed that microcytosis and hypochromia were common in both IDA and thalassemia, with no statistically significant difference. This finding indicates that both disorders commonly present as microcytic hypochromic anemia and cannot be reliably differentiated by these two features alone. However, anisocytosis and pencil cells were significantly more common in IDA, while target cells, basophilic stippling, nucleated RBCs, tear drop cells, and polychromasia were significantly more frequent in thalassemia. Chaichompoo et al. (2019)[3] observed that thalassemia patients frequently demonstrated abnormal RBC morphology including target cells, poikilocytosis, anisocytosis, and oxidative stress-related morphological changes. Körber et al. (2017)[6] described target cells and marked microcytosis as characteristic features of β-thalassemia minor. Ahmad et al. (2018)[11] reported that morphological analysis of erythrocytes, particularly evaluation of target cells, anisopoikilocytosis, and elliptocytes, can improve differentiation between IDA and thalassemia. Wang et al. (2021)[4] further demonstrated that automated RBC morphological analysis software could effectively distinguish thalassemia from iron deficiency anemia based on characteristic red cell abnormalities.

The present study also showed that diagnostic morphological patterns were useful in distinguishing the two conditions. Predominant anisopoikilocytosis, marked hypochromia with low RBC count, pencil/elliptocyte predominance, and high RDW pattern were significantly associated with IDA. In contrast, microcytosis disproportionate to anemia, target cell predominance, basophilic stippling predominance, and relatively preserved or increased RBC count were strongly associated with thalassemia. Pengon et al. (2018)[10] reported that thalassemia patients frequently exhibit marked morphological abnormalities including target cells, basophilic stippling, and anisopoikilocytosis, particularly when associated with oxidative stress or coexisting hematological disorders. Ford et al. (2013)[1] also emphasized that peripheral smear examination remains an essential component in the diagnostic evaluation of anemia despite advances in automated hematology analyzers. Wang et al. (2021)[4] similarly concluded that integration of hematological indices with RBC morphology improves diagnostic accuracy in distinguishing IDA from thalassemia.

Conclusion

The present cross-sectional study demonstrated significant differences in red blood cell morphology and hematological parameters between iron deficiency anemia (IDA) and thalassemia patients. Although both conditions commonly presented as microcytic hypochromic anemia, important distinguishing features were identified on peripheral blood smear examination and routine hematological analysis. Patients with iron deficiency anemia showed significantly higher red cell distribution width (RDW), lower RBC count, marked anisopoikilocytosis, and predominance of pencil cells and elliptocytes. In contrast, thalassemia patients demonstrated significantly lower MCV, relatively preserved or increased RBC count, and higher frequency of target cells, basophilic stippling, nucleated red blood cells, and polychromasia.

The study findings highlight that peripheral smear examination remains a valuable, simple, and cost-effective diagnostic tool in differentiating iron deficiency anemia from thalassemia, especially in resource-limited settings where advanced diagnostic facilities may not be readily available. Careful assessment of RBC morphology in conjunction with hematological indices such as MCV, RDW, RBC count, and serum ferritin can improve diagnostic accuracy and assist clinicians in early diagnosis and appropriate management.

The study also emphasizes that no single morphological feature alone is sufficient for definitive diagnosis; rather, a combination of characteristic findings should be interpreted collectively. The significant association of target cells and basophilic stippling with thalassemia and the predominance of anisopoikilocytosis and pencil cells in IDA can serve as important supportive diagnostic clues in routine clinical practice.

Overall, the present study concludes that comparative evaluation of red blood cell morphology provides meaningful diagnostic insight and plays an important role in distinguishing iron deficiency anemia from thalassemia. Integration of peripheral smear findings with clinical and laboratory parameters may help reduce diagnostic confusion, avoid inappropriate therapy, and facilitate timely treatment and genetic counseling where necessary.

LIMITATIONS OF THE STUDY

1. The study was conducted at a single tertiary care center, which may limit the generalizability of the findings to the wider population.
2. The sample size was relatively limited and may not represent all variants of thalassemia and iron deficiency anemia.
3. Being a cross-sectional study, long-term follow-up and progression of hematological changes could not be assessed.
4. Molecular studies and genetic analysis for confirmation of thalassemia subtypes were not performed in all patients.
5. Interobserver variation in interpretation of peripheral smear morphology may have influenced some findings.
6. Serum ferritin levels may be affected by inflammatory conditions, which could alter assessment of iron status in certain patients.
7. Coexisting nutritional deficiencies such as vitamin B12 or folate deficiency were not extensively evaluated.
8. Advanced automated morphological analysis techniques were not utilized in the present study.
9. The study did not include correlation with bone marrow findings.
10. Peripheral smear morphology may overlap in some cases, making differentiation difficult in borderline presentations.

References

1. Ford J. Red blood cell morphology. International journal of laboratory hematology. 2013 Jun;35(3):351-7.
2. Jassim AN. Comparative behavior of red blood cells indices in iron deficiency anemia and β-thalassemia trait. Iraqi Journal of Hematology. 2016 Jul 1;5(2):183-6.
3. Chaichompoo P, Qillah A, Sirankapracha P, Kaewchuchuen J, Rimthong P, Paiboonsukwong K, Fucharoen S, Svasti S, Worawichawong S. Abnormal red blood cell morphological changes in thalassaemia associated with iron overload and oxidative stress. Journal of Clinical Pathology. 2019 Aug 1;72(8):520-4.
4. Wang F, Wang G, Yang Z, Wang X, Liu D, Wan N, Wu W. Differential diagnosis of thalassemia and iron deficiency anemia using the CellaVision Advanced Red Blood Cell software. International Journal of Laboratory Hematology. 2021 Aug;43(4):853-8.
5. Sun A, Chang JY, Jin YT, Chiang CP. Differential diagnosis between iron deficiency anemia and thalassemia trait-induced anemia. Journal of Dental Sciences. 2023 Oct 1;18(4):1963-4.
6. Körber C, Wölfler A, Neubauer M, Robier C. Red blood cell morphology in patients with β-thalassemia minor. LaboratoriumsMedizin. 2017 Feb 1;41(1):49-52.
7. Jameel T, Baig M, Ahmed I, Hussain MB, bin Doghaim Alkhamaly M. Differentiation of beta thalassemia trait from iron deficiency anemia by hematological indices. Pakistan journal of medical sciences. 2017 May;33(3):665.
8. Ayyıldız H, Tuncer SA. Determination of the effect of red blood cell parameters in the discrimination of iron deficiency anemia and beta thalassemia via Neighborhood Component Analysis Feature Selection-Based machine learning. Chemometrics and Intelligent Laboratory Systems. 2020 Jan 15;196:103886.
9. Lei MQ, Sun LF, Luo XS, Yang XY, Yu F, Chen XX, Wang ZM. Distinguishing iron deficiency anemia from thalassemia by the red blood cell lifespan with a simple CO breath test: a pilot study. Journal of breath research. 2019 Apr 1;13(2):026007.
10. Pengon J, Svasti S, Kamchonwongpaisan S, Vattanaviboon P. Hematological parameters and red blood cell morphological abnormality of Glucose-6-Phosphate dehydrogenase deficiency co-inherited with thalassemia. Hematology/oncology and stem cell therapy. 2018 Jan 1;11(1):18-24.
11. Ahmad I, Abdullah SN, Sabudin RZ. Morphological features analysis for erythrocyte classification in IDA and thalassemia. International Journal of Advanced Computer Science and Applications. 2018;9(12).
12. Düzenli Kar Y, Altınkaynak K. Reticulocyte hemoglobin equivalent in differential diagnosis of iron deficiency, iron deficiency anemia and β thalassemia trait in children. Turkish Journal of Biochemistry. 2021 Feb 26;46(1):45-51.