Original Article

Detection of Latent Iron Deficiency and Thalassemia Trait with Red Cell Indices and Peripheral Blood Smear

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ABSTRACT

Objective: To evaluate the effectiveness of red cell indices and peripheral blood smear findings in detecting latent iron deficiency and differentiating it from thalassemia trait, and to determine the diagnostic accuracy of commonly used discrimination indices in distinguishing between these two causes of microcytosis.

Study Design: Observational cross-sectional study

Place and Duration of Study: This study was conducted at the Department of Pathology (Hematology) at Pakistan Railway Hospital (PRH), in collaboration with the Medicine, Surgery, and Gynecology/Obstetrics departments. The study was carried out over one year, from September 2023 to September 2024.

Methods: This study was conducted over one year and involved 285 adults referred from outpatient departments in medicine and surgery, with haemoglobin levels ranging from 10 to 12 g/dL. Participants included both males and females, with a predominant representation of young adults. Data collection focused on complete blood counts, red cell indices, and blood smear examinations.

Results: A total of 285 samples were collected and analyzed, comprising 50.9% male and 49.1% female participants, with a mean age of 24±3 years. Haematological parameters, including haemoglobin, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and red cell distribution width (RDW), were recorded. The findings revealed that 61.4% of participants were diagnosed with latent iron deficiency, while 38.6% were identified as having thalassemia trait on the basis of CBC and blood smears.

Conclusion: The data analysis revealed a high prevalence of latent iron deficiency, characterized by a hypochromic microcytic blood picture and low ferritin levels. In contrast, participants with thalassemia trait also exhibited microcytosis but typically had normal or elevated ferritin levels. Despite similar haematological features, the conditions stem from different causes.

Key Words: Iron, Anemia, Thalassemia, Peripheral, Blood smear, MCH, Microcytosis, Blood

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INTRODUCTION

Iron deficiency is the most prevalent nutritional deficiency worldwide, affecting a significant portion of the population, particularly in developing countries[1]. It occurs when there is inadequate iron to meet the body's needs, leading to reduced hemoglobin synthesis and anemia. Thalassemia, on the other hand, is a genetic disorder characterized by abnormal hemoglobin production[2].

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The thalassemia trait, or carrier state, often presents with mild anemia and microcytosis, which can be easily confused with iron deficiency anemia. Both conditions share similar hematological features, making differential diagnosis challenging but essential for appropriate treatment[3]. Prevalence rates of iron deficiency do not vary much between developed and developing nations, with young women, infants, and children being the most affected populations[4]. Latent iron deficiency, characterized by iron deficiency without anemia, often goes unnoticed but can have significant health implications. Latent iron deficiency is marked by a serum ferritin level of ≤15 μg/L and hemoglobin >11 g/dL[5]. Iron deficiency anemia ensues when hemoglobin drops to ≤12 g/dL alongside low serum ferritin. Low serum ferritin levels (<12 ng/mL) are specific indicators of iron deficiency[6]. A complete blood count (CBC) might reveal a normal hemoglobin level in latent iron deficiency but indicate a decrease in serum ferritin levels, suggesting a depletion of iron stores in the body. Peripheral blood smear findings in latent iron deficiency may include anisopoikilocytosis

with hypochromia and microcytosis[7]. Occasional target cells may be present, indicating early changes in red cell morphology. This can lead to more severe health issues such as impaired cognitive and physical development in children and reduced work capacity in adults[8]. Latent iron deficiency is a stage when the body's iron stores are depleted, yet hemoglobin levels have not fallen below the threshold for anemia. This stage is critical as it can still affect various bodily functions and overall health[9]. Iron is a vital component of hemoglobin, which is necessary for oxygen transport in the blood. Even without anemia, low iron levels can lead to fatigue, reduced physical performance, and impaired immune function. Iron is crucial for cognitive development and function, and latent iron deficiency in children can result in long-term developmental deficits[10]. The diagnostic approach for detecting latent iron deficiency involves assessing clinical symptoms and laboratory parameters. Symptoms may include fatigue, pallor, and weakness. However, these are non-specific and can also be present in other conditions[11]. Laboratory tests are more definitive, with serum ferritin being the most reliable indicator of iron status. Ferritin reflects the body's iron stores, and low levels indicate depletion. However, ferritin is also an acute-phase reactant and can be elevated in the presence of inflammation, infection, or liver disease, complicating the diagnosis[12]. Other tests such as transferrin saturation and serum iron levels can provide valuable information about iron status. Transferrin saturation, which measures the percentage of transferrin that is saturated with iron, is typically low in iron deficiency[13]. Serum iron levels can also be low, but they are subject to diurnal variation and influenced by recent dietary intake, making them less reliable as a standalone test[14]. The treatment of latent iron deficiency focuses on replenishing iron stores and addressing the underlying cause. Oral iron supplements are commonly used to increase serum ferritin and hemoglobin levels. Ferrous sulfate is the most widely used preparation, although other forms, such as ferrous gluconate and fumarate, are also available[15].

METHODS

This observational cross-sectional study was conducted in the Department of Pathology (Hematology) at Pakistan Railway Hospital (PRH), in collaboration with the Medicine, Surgery, and Gynecology/Obstetrics departments. The study was carried out over one year, from September 2023 to September 2024, following approval from the Institutional Review Committee of Riphah International University, Islamabad. A sample size of 285 was determined using Cochran's formula based on a 25% prevalence estimate, a 95% confidence level, and a 5% margin of error. Participants were selected through non-probability convenience sampling. Adult male and female patients referred from outpatient departments, with hemoglobin levels between 10–12 g/dl, were included. Individuals with chronic systemic disease, those receiving hematinic therapy, or those who had undergone recent blood transfusion were excluded. Non-probability convenience sampling was employed.

Inclusion Criteria

- Adult male and female patients referred from Medicine, Surgery, and Gynecology/Obstetrics OPDs
- Hemoglobin level between 10–12 g/dl on CBC

Exclusion Criteria

- History of chronic systemic disease
- Current use of hematinic therapy
- History of recent blood transfusion

Data Collection: A total of 285 adult patients meeting the inclusion criteria were enrolled. After obtaining informed consent while ensuring anonymity and confidentiality, detailed clinical history and physical examination findings were recorded using a structured proforma. Blood samples were collected from all eligible participants for hematological analysis. Complete blood counts (CBC) were performed using the Mindray BC-5000 analyzer. Each sample was matched with patient details, gently inverted 10–20 times for homogenization, scanned for identification, and aspirated automatically by the analyzer. Printed results were reviewed, abnormal parameters highlighted, and attached to patient records. Peripheral blood smears were prepared by placing a 4 mm blood drop on a clean glass slide, spreading it at a 45° angle, and allowing it to air dry. Slides were labeled with a lead pencil, flooded with Leishman stain for five minutes, followed by buffered water for 10 minutes. Slides were washed, dried, and examined under 10×, 40×, and 100× objectives. Morphological features such as microcytosis, hypochromia, anisopoikilocytosis, pencil cells, target cells, and basophilic stippling were noted. Any diagnostic uncertainty was discussed with the hematologist before final reporting. Serum ferritin levels were assessed using the Rayto RT-6000 microplate ELISA reader. Two milliliters of blood were centrifuged at 5000 rpm for five minutes to obtain serum. Wash buffer was prepared by mixing 25 ml concentrate with 475 ml distilled water. Ferritin standards, controls, and patient samples (25 µl each) were pipetted into microplate wells, followed by 100 µl enzyme conjugate. After a 30-minute incubation, the wells were washed three times with buffer, blotted dry, and then incubated with 100 µl TMB substrate for 15–30 minutes in the dark. A stop solution (50 µl) was added, and absorbance was measured at 450 nm. Ferritin levels were calculated by comparing absorbance readings with the standard curve. Based on CBC parameters, peripheral smear morphology, and ferritin levels, participants were classified into two

diagnostic groups: latent iron deficiency and thalassemia trait.

Data Analysis: Data were entered and analyzed using SPSS version 27. Continuous variables were expressed as mean ± standard deviation (SD). Categorical variables were analyzed using the Chi-square test. A p-value < 0.05 was considered statistically significant. To maintain data quality, strict adherence to standard operating procedures (SOPs) was ensured throughout collection, transport, analysis, and storage. All samples were examined for hemolysis, clot formation, adequate volume, appropriate labeling, and correct timing of collection. Analyzer performance was routinely validated using hematology cell controls.

RESULTS

Data were collected from 285 patients. The sample comprised almost equal proportions of males (50.9%) and females (49.1%), with a mean age of 24.10 ± 3.43 years. Nearly half of the participants were students (44.9%), while 18.6% were employed in private jobs and 36.5% were unemployed.

Table No. 1. Combined Demographic, Clinical, and Laboratory Characteristics of Study Participants (n = 285)

Variable	Category / Statistic	n (%) / Mean ± SD
Gender	Male	145 (50.9%)
	Female	140 (49.1%)
Age (years)	Mean ± SD	24.10 ± 3.43
Occupation	Student	128 (44.9%)
	Private job	53 (18.6%)
	Unemployed	104 (36.5%)
Weight (kg)	Mean ± SD	70.51 ± 10.88
Hemoglobin (g/dl)	Mean ± SD	10.61 ± 0.63
WBC count (10^9/L)	Mean ± SD	7.42 ± 2.07
RBC count (mcl)	Mean ± SD	4.23 ± 0.84
MCV (fL)	Mean ± SD	73.89 ± 4.59
MCH (pg)	Mean ± SD	24.22 ± 1.99
RDW (%)	Mean ± SD	13.04 ± 1.17
Platelet count (10^9/L)	Mean ± SD	296.64±86.32
Ferritin (ng/mL)	Mean ± SD	49.43 ± 40.98

The mean body weight was 70.51 ± 10.88 kg. Hematological indices showed a mean hemoglobin level of 10.61 ± 0.63 g/dL, consistent with mild anemia. The mean RBC count was 4.23 ± 0.84 mcl, with accompanying microcytosis (MCV 73.89 ± 4.59 fL) and low MCH (24.22 ± 1.99 pg). RDW levels showed mild anisocytosis (13.04 ± 1.17%), while platelet counts remained within the normal range (296.64 ± 86.32 × 10^9/L). Mean serum ferritin was 49.43 ± 40.98 ng/mL, indicating variable iron stores across participants. No significant correlation was observed between gender and hemoglobin (r = .004, p = .950). Hemoglobin showed a strong, statistically significant positive correlation with RBC count (r = .726, p < .001), suggesting that higher red cell mass corresponded with improved hemoglobin concentration. A moderate positive correlation was also noted between hemoglobin and ferritin levels (r = .487, p < .001), indicating that individuals with greater iron stores generally demonstrated higher hemoglobin values.

Table No. 2. Combined Correlation Matrix of Gender, Hemoglobin, RBC Count, and Ferritin Levels (n = 285)

Variables	Gender	Hb (g/dl)	RBC Count (mcl)	Ferritin (ng/mL)
Gender	Pearson Correlation	1	.004	—
	Sig. (2-tailed)	—	.950	—
	N	285	285	285
Hb (g/dl)	Pearson Correlation	.004	1	.726**
	Sig. (2-tailed)	.950	—	< .001
	N	285	285	285
RBC Count (mcl)	Pearson Correlation	—	.726**	1
	Sig. (2-tailed)	—	< .001	—
	N	285	285	285
Ferritin (ng/mL)	Pearson Correlation	—	.487**	—
	Sig. (2-tailed)	—	< .001	—
	N	285	285	285

Table No. 3: Association Between Peripheral Smear Findings and Final Classification (n = 285)

Peripheral Smear Findings	Latent Iron Deficiency (n)	Thalassemia Trait (n)	Total (n)	% Within Category
Microcytosis, Hypochromia, Anisocytosis, Poikilocytosis	175	0	175	100% in LID / 0% in TT
Hypochromia, Microcytosis, Anisopoikilocytosis, Target cells, Occasional Fragmentation	0	110	110	0% in LID / 100% in TT
Total	**175**	**110**	**285**	61.4% LID / 38.6% TT

All 175 individuals (100 percent) classified as latent iron deficiency exhibited microcytosis, hypochromia, anisocytosis, and poikilocytosis. In contrast, all 110 individuals (100 percent) diagnosed with thalassemia trait showed hypochromia, microcytosis, anisopoikilocytosis, target cells, and occasional fragmentation. There was no overlap in smear morphology between the groups. Overall, 61.4 percent (n = 175) of the study sample was categorized as latent iron deficiency, while 38.6 percent (n = 110) was classified as thalassemia trait. The association was not statistically significant ($\chi^2 = 0.245$, df = 1, p = 0.620). Similar findings were observed with the continuity correction test (0.140, p = 0.709), likelihood ratio (0.245, p = 0.620), and Fisher's Exact test (p = 0.629). The linear-by-linear association showed 0.244 with a p-value of 0.621. All 285 cases were valid for analysis.

Table No. 4: Chi-Square Test for Association Between Peripheral Smear Pattern and Diagnostic Classification

Test	Value	df	p-value (2-sided)
Pearson Chi-Square	0.245	1	0.620
Continuity Correction	0.140	1	0.709
Likelihood Ratio	0.245	1	0.620
Fisher's Exact Test	—	—	0.629
Linear-by-Linear Association	0.244	1	0.621
Valid Cases	**285**	—	—

DISCUSSION

Our study was conducted to evaluate and determine the detection of latent iron deficiency & Thalassemia trait with red cell indices and peripheral blood smear. A total of 285 samples were collected and analyzed. Out of 285 participants, the male participants were 50.9% while the females were 49.1%. The mean age of participants recorded was 24±3 years. In our study the majority of population belong to the age group of 21-25 years with minimum age was 18 years and the maximum was 29 years. Out of 285, 122 participants belonged to age group ranging from 21-25 years. A study conducted a study on 620 participants with majority of participants were from the age range of 21-36 years. Similar to our study, all the participants underwent serum iron and ferritin with individuals showing low hemoglobin. All those participants underwent morphology with MCV, RBC count and red cell distribution width index. The study concluded a total of 135 individuals with hypochromic microcytic anemia having the normal hemoglobin F and hemoglobin A2 <3.2. out of it, 93 participants were diagnosed with iron deficiency anemia and 32 with Beta thalassemia trait. As compared to the iron deficiency anemia, the RBC count was relatively higher and MCV was much lower in beta thalassemia trait patients[16]. In a nutshell, the RDWI (Red cell distribution width index was a reliable index in differentiating the iron deficiency with the Beta Thalassemia trait. In our study, different parameters were recorded such as Hemoglobin, mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH) and Red cell distribution width (RDW). The mean hemoglobin calculated in our patients was 10.61 mg/dl and MCV was reported to be 73.89fl which interprets that the participants have hypochromic and microcytic blood picture[17]. Moreover, the RDW of 13.04% indicates the variability of red cell size which supports the evidence that the participants were suffering from the iron deficiency. Furthermore, the participants reported with low ferritin level of 49.43 ng indicates more of a latent iron deficiency[18]. The thalassemia trait is distinguished from iron deficiency by normal or high ferritin levels, even in the presence of microcytic hypochromic blood findings. While low MCV with normal or high ferritin indicates thalassemia trait, low MCV and low ferritin alone is more suggestive of iron deficiency[19].

In another study conducted by on 131 individuals who underwent RBC indices such as RBC count, RDWI, MCV and MCH along with different index such as Mentzer index, Shine and Lal index, sensitivity, specificity, positive & negative predictive values and compared them with cutoff values available in the literature. As a result, all the values were reported to be higher than the cutoff values indicating 50 participants diagnosed with Iron deficiency anemia and Beta thalassemia trait and alpha thalassemia trait in 31 participants. In our study, the mean of Hemoglobin levels was calculated with a low range of 10.61 g/dl indicating the majority of population as suffering from hypochromic microcytosis[20-22]. The WBC count and platelet count were reported to be in the normal limit hence indicating no association in diagnosing the iron deficiency and thalassemia trait[23]. This study has several limitations that should be considered when interpreting the findings. The use of non-probability convenience sampling limits the generalizability of the results to the broader population. The cross-sectional design captures hematological parameters at a single point in time, preventing assessment of temporal changes or progression from latent iron deficiency to overt anemia. Serum ferritin levels, although useful, may be influenced by inflammation or subclinical infection, which was not controlled through additional biomarkers such as CRP. The study also relied heavily on peripheral smear interpretation, which, despite being cross-checked with a hematologist, is subject to observer variability.

CONCLUSION

The analysis of the data reveals a significant prevalence of latent iron deficiency among the study population, characterized by a hypochromic microcytic blood picture and low ferritin levels. In contrast, participants

classified with thalassemia trait also exhibited microcytic red blood cells but typically have normal or elevated ferritin levels. While both conditions share similar hematological features, they arise from distinct underlying causes related to iron metabolism. The findings underscore that latent iron deficiency, a major health problem in society, requires greater attention due to its widespread nature and potential long-term impact.

Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Sana Saleem Rana, Ayesha Nayyar, Rahmat Javed
Drafting or Revising Critically:	Mehak Mohsin, Sehrish Salman, Amna Javed
Final Approval of version:	All the above authors
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