Original article

Research on the relationship between markers of iron metabolism in patients with hypochromic microcytic anemia

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Abstract

Background: Hypochromic microcytic anemia (HMA) is a common condition with serious consequences if not detected and treated promptly. Hematological tests and biochemical indicators assessing iron metabolism help to identify the causes of HMA, especially iron deficiency anemia (IDA), hemoglobinopathy (HGP) and anemia of chronic disease (ACD).

Objective: To determine several indicators assessing iron metabolism in HMA.

Subjects and methods: A cross-sectional study was conducted on 168 patients with HMA who visited and received treatment at Hue University of Medicine and Pharmacy Hospital from January 2025 to January 2026, with three groups of causes including IDA, HGP and ACD.

Results: The clinical manifestations of HMA were diverse. The most common symptom was pallor (48.2%), followed by fatigue (47.6%), anorexia (31.0%), dyspnea (19.0%). The cut-off values of serum iron, ferritin, transferrin saturation (TfS), unsaturated iron binding capacity (UIBC) in the prediction of HMA were 47.7 µg/dL, 112.7 µg/L, 13.7%, 50.0 µmol/L, respectively. The cut-off values of transferrin and TfS in the prediction of HGP were 274.5 mg/dL and 6.5%, respectively. Transferrin was an independent predictor of HGP and ACD, while serum iron and TfS were independent predictors of ACD.

Conclusions: Patients with HMA exhibited heterogeneous clinical manifestations. Serum iron, ferritin, TfS, and UIBC were useful predictors of HMA. Transferrin independently predicted HGP and ACD, whereas serum iron and TfS independently predicted ACD.

Key words: *Hypochromic microcytic anemia, iron deficiency anemia, hemoglobinopathy, anemia of chronic disease.*

1. INTRODUCTION

Anemia constitutes a significant global public health concern, disproportionately affecting adolescent girls, women of reproductive age (15–49 years), pregnant women, and young children, particularly in low- and middle-income countries. According to the World Health Organization (WHO), in 2019 approximately 30% (571 million) of women aged 15–49 years, 37% (32 million) of pregnant women, and 39.8% (269 million) of children aged 6–59 months were affected by anemia. The highest prevalence rates were observed in the African and Southeast Asian regions [1]. There are many different causes of anemia, manifesting as various types of anemia clinically, among which HMA accounts for a relatively high percentage. There are five major clinical causes of HMA with IDA, HGP, ACD, sideroblastic anemia, and lead poisoning. Among these, sideroblastic anemia and lead poisoning are relatively uncommon and, in the case of sideroblastic anemia, often require invasive diagnostic procedures. Accurate differentiation among these etiologies is essential, as management strategies, monitoring approaches, and patient counseling vary substantially. Whole blood parameters and markers of iron metabolism play a critical role in distinguishing between these forms of anemia. Therefore, we conducted this research with two aims:

1. To describe the clinical and paraclinical characteristics of patients with hypochromic microcytic anemia.

2. *To investigate the predictive value of several indicators assessing iron metabolism in the causes of hypochromic microcytic anemia.*

2. SUBJECTS AND METHODS

2.1. Study design: A cross-sectional study was carried out on patients with HMA from January 2025 to January 2026 at Hue University of Medicine and Pharmacy Hospital, in Hue City, Vietnam.

2.2. Study sampling

Patient group: satisfy the following criteria

- Age: ≥ 18 years old.
- Hypochromic microcytic anemia:

+ Anemia (Figure 1)

Table 1. WHO classification of anemia according to age and severity based on Hb (g/L) [2]

Population	Non-anemia	Mild anemia	Moderate anemia	Severe anemia
6-59 months of age	≥ 110	100 - 109	70 - 99	< 70
5-11 years of age	≥ 115	110 - 114	80 - 109	< 80
12-14 years of age	≥ 120	110 - 119	80 - 109	< 80
Non-pregnant women (≥ 15 years)	≥ 120	110 - 119	80 - 109	< 80
Pregnant women	≥ 110	100 - 109	70 - 99	< 70
Men (≥ 15 years)	≥ 130	110 - 129	80 - 109	< 80

Hb: Hemoglobin

+ Hypochromic and microcytic erythrocyte: MCV < 80 fL and MCH < 28 pg.

+ The patient provided informed consent to participate in the study.

Exclusion criteria:

- Anemia due to acute blood loss.
- Metastatic cancer invading the bone marrow.
- Hematological malignancies.
- Subjects were treated with iron supplements by healthcare facilities.

Control group

- Age: ≥ 18 years old.
- No hypochromic microcytic anemia.
- Provided informed consent to participate in the study.
- Subjects were not supplemented with iron products.

2.3. Measurements

- Four mililiters of whole blood were collected from each study subject into EDTA (2 mL) (for complete blood count (CBC) test and hemogram) and heparin (2 mL) tubes (for serum iron, ferritin, and transferrin tests) .

- The CBC was analyzed using a Sysmex XN-550 automated hematology analyzer, and the morphology of the blood cells was analyzed under a light microscope at the Department of Hematology and Transfusion, Hue University of Medicine and Pharmacy Hospital.

- Serum iron, ferritin, and transferrin tests were analyzed using a Cobas-8000 automated analyzer at the Department of Biochemistry-Immunology, Hue University of Medicine and Pharmacy Hospital.

- Transferrin saturation (TfS) was calculated using the formula of Steven M. Truscott (2020): TfS = x 0,673 x 100% [3].

- The TIBC, UIBC were calculated indirectly using the formula of Ishmael Kasvosve and Joris Delanghe (2002) [4]:

TIBC (total iron binding capacity) (µmol/L) = transferrin (g/L) x 25.2

UIBC (µmol/L) = TIBC (µmol/L) – serum iron (µmol/L)

- Etiological investigation of HMA reveals three main categories of underlying causes, namely:

+ Iron deficiency anemia (IDA): Laboratory tests show serum iron and ferritin levels < 30 ng/mL or transferrin saturation < 30% [5].

+ Hemoglobinopathy (HGP)

- Alpha thalassemia: Hemoglobin component test shows Hb Bart's and/or HbH.
- Beta thalassemia: Hemoglobin component test shows increased HbA2 (> 3.5%) and/or increased HbF.
- Hemoglobin abnormalities:

➢ Hemoglobin abnormalities (α-globin chain): Hemoglobin component test shows HbCs or Hb Quong Sze (HbQs), Hb Pakse'.

➢ Hemoglobin abnormalities (β-globin chain): Hemoglobin component test shows HbS (sickle cell) or HbE, HbC [5].

+ Anemia of chronic disease (ACD): chronic diseases (chronic inflammation and infection, benign and malignant tumors, autoimmune diseases, congestive heart failure, chronic kidney disease) with presence of anemia, transferrin saturation < 16%, serum ferritin > 100 ng/mL [6].

A total of 68 subjects were included in the control group and 168 subjects in the patient group, including 101, 34, and 33 patients with IDA, HGP, and ACD, respectively.

2.4. Data analysis

Data was entered and managed using Microsoft

Excel 365 version 2010, and analyzed using SPSS version 26.0. Descriptive statistics: Qualitative variables were described using quantities and percentages; quantitative variables were described using mean and standard deviation. Receiver operating characteristic (ROC) curve analysis was performed to evaluate the diagnostic performance of the model. The area under the ROC curve (AUC) was calculated to assess the overall discriminative ability. The optimal cut-off values were determined using the Youden index (sensitivity + specificity − 1), selecting the value that maximized both sensitivity and specificity. $P < 0.05$ was considered statistically significant.

2.5. Ethical considerations

All participants gave their informed consent. We received the permission paper from the Ethical Committee of University of Medicine and Pharmacy, Hue University (number H2025/313).

3. RESULTS

3.1. Clinical characteristics

Table 2. Frequency of clinical characteristics among patients with HMA

Clinical characteristics	n	%
Dyspnea	32	19.0%
Palpitations	14	8.3%
Pallor	81	48.2%
Brittle nails	9	5.4%
Alopecia	7	4.2%
Dizziness	29	17.3%
Headache	3	1.8%
Fatigue	80	47.6%
Anorexia	52	31.0%
Hepatomegaly	17	10.1%
Splenomegaly	9	5.4%
Jaundice	3	1.8%

The clinical manifestations of HMA were diverse. The most common symptom was pallor (48.2%), followed by fatigue (47.6%), anorexia (31.0%), dyspnea (19.0%).

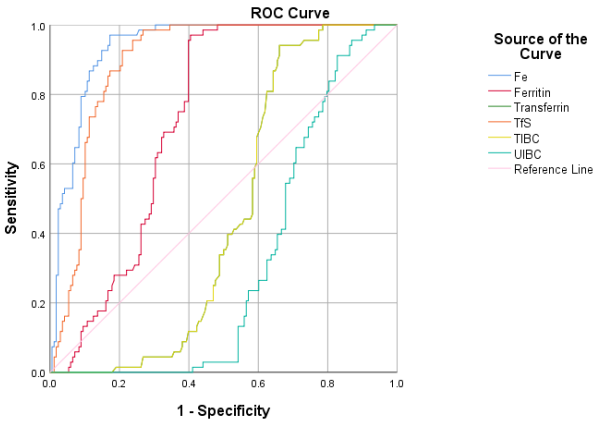
3.2. Characteristics of red blood cells morphology

Table 3. Morphological characteristics of red blood cells in the study subjects

		IDA (n = 101) (%)	HGP (n = 34) (%)	ACD (n = 33) (%)	p
Anisocytosis		95 (94.1%)	32 (94.1%)	32 (97.0%)	0.803
Reticulocytosis		3 (3.0%)	24 (70.6%)	6 (18.2%)	**< 0.001**
Morphology	**Annulocytes**	90 (89.1%)	32 (94.1%)	27 (81.8%)	0.277
	Target cells	11 (10.9%)	28 (82.4%)	10 (30.3%)	**< 0.001**
	Pencil cells	45 (44.6%)	11 (32.4%)	10 (30.3%)	0.226
	Tear drop cells	27 (26.7%)	15 (44.1%)	8 (24.2%)	0.118
	Acanthocyte	3 (3.0%)	2 (5.9%)	1 (3.0%)	0.718
	Stomatocyte	7 (6.9%)	6 (17.6%)	1 (3.0%)	0.069
	Sickle cell	1 (1.0%)	1 (2.9%)	0 (0.0%)	0.517
	Fragment	0 (0.0%)	3 (8.8%)	2 (6.1%)	**0.016**

There were statistically significant differences between the three disease groups regarding the increase in reticulocytes, target cells, and fragments (predominant in the HGP group).

3.3. Cut-off values in the prediction of hypochromic microcytic anemia



	AUC	Cut-off	Sensitivity (Se)	Specificity (Sp)	95% CI	p
Serum iron (µg/dL)	0.937	47.7	97.1%	75.0%	0.907-0.966	**< 0.001**
Ferritin (µg/L)	0.726	112.7	75.0%	61.9%	0.665-0.788	**< 0.001**
Transferrin (mg/dL)	0.455	264.5	36.8%	48.8%	0.384-0.525	0.276
TfS (%)	0.894	13.7	98.5%	69.6%	0.854-0.934	**< 0.001**
TIBC (µmol/L)	0.455	66.7	36.8%	48.8%	0.384-0.525	0.276
UIBC (µmol/L)	0.313	50.0	60.1%	75.0%	0.248-0.378	**< 0.001**

Figure 1. Indicators in the prediction of HMA

The cut-off values of serum iron, ferritin, TfS, UIBC in the prediction of HMA were 47.7 µg/dL, 112.7 µg/L, 13.7%, 50.0 µmol/L, respectively.

3.4. Assessing iron metabolism indices for IDA

Table 4. Multivariate logistic regression model of predictor factors for IDA

Index	OR	95%CI	p
Serum iron	0.831	0.000-1.083E+89	0.999
Ferritin	0.693	0.000-2.033E+10	0.976
Transferrin	1.063	0.000-7.241E+38	0.999
TfS	1.711	0.000-1.780E+297	0.999

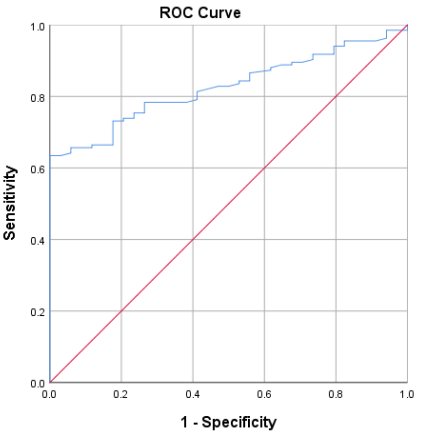
There were no iron metabolism assessment tests that predict IDA in HMA patients.

3.5. Assessing iron metabolism indices for HGP

Table 5. Multivariate logistic regression model of predictor factors for HGP

Index	OR	95%CI	p
Serum iron	1.035	0.986-1.087	0.167
Ferritin	1.000	0.999-1.000	0.545
Transferrin	0.980	0.966-0.993	0.004
TfS	0.991	0.854-1.150	0.907

Transferrin was an independent predictor of HGP in HMA patients.



	AUC	Cut-off	Sensitivity (Se)	Specificity (Sp)	95% CI	p
Transferrin (mg/dL)	0.826	274.5	61.2%	100%	0.765 - 0.887	< 0.001

Figure 2. Transferrin in HGP prediction compared to (IDA+ACD)

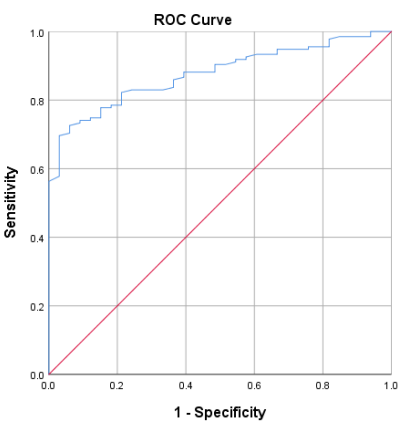
The cut-off value of transferrin in the prediction of HGP was 274.5 mg/dL.

3.6. Assessing iron metabolism indices for ACD

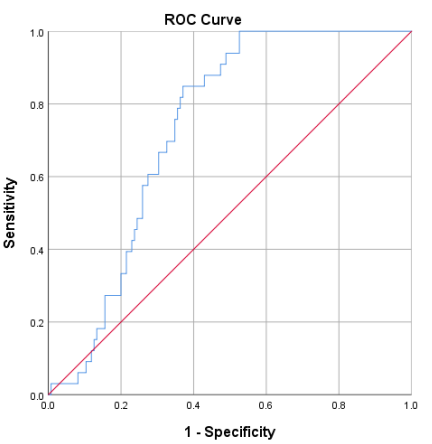
Table 6. Multivariate logistic regression model of predictor factors for ACD

Index	OR	95%CI	p
Serum iron	1.089	1.032-1.150	**0.002**
Ferritin	1.001	1.000-1.002	0.171
Transferrin	0.961	0.946-0.977	**< 0.001**
TfS	0.748	0.625-0.895	**0.001**

Serum iron, transferrin and TfS were independent predictors of ACD in HMA patients.



Transferrin



TfS

	AUC*	Cut-off	Sensitivity (Se)	Specificity (Sp)	95% CI	p
Transferrin (mg/dL)	0.877	274.5	60.0%	70.0%	0.824 - 0.929	**< 0.001**
TfS (%)	0.734	6.5	84.8%	58.5%	0.660 - 0.807	**< 0.001**

Figure 3. Transferrin in ACD prediction compared to (IDA+HGP)

The cut-off values of transferrin and TfS for predicting ACD were 274.5 mg/dL and 6.5%, respectively.

4. DISCUSSION

The three most common causes of HMA are IDA, HGP, and ACD. These conditions share the common feature of anemia syndrome, with largely overlapping clinical manifestations. Symptom severity varies depending on the degree of anemia, the patient's physiological adaptation, and response to treatment. In the disease group, clinical manifestations varied widely, ranging from functional symptoms such as fatigue (47.6%), anorexia (31.0%), dizziness (17.3%), dyspnea (19.0%), and palpitations (8.3%) which were the primary reasons for seeking medical care to physical symptoms, including pallor of the skin and mucous membranes (48.2%), hepatomegaly (10.1%), splenomegaly (5.4%), brittle nails (5.4%), and jaundice (1.8%) (Table 2). A domestic study conducted by Tran Xuan Tuan et al. (2022) reported that pallor of the skin and mucous membranes was the most prevalent clinical sign, accounting for 91.6% of cases [7]. Similarly, an international study by Fathy H.A. demonstrated that pallor was the most common clinical manifestation among patients with HMA [8]. Notably, a substantial proportion of patients are hospitalized without overt symptoms of anemia and are subsequently diagnosed with HMA during routine health examinations or while being treated for other conditions. This underscores the importance of community-based screening for HMA, particularly in regions with a high prevalence of anemia, such as Vietnam.

Analysis of peripheral blood smear analysis in patients with HMA demonstrated statistically significant differences in reticulocytosis, the presence of target cells, and RBC fragments among the three etiological groups ($p < 0.05$) (Table 3). These abnormalities were most pronounced in the HGP group and represent key hematological features that may assist clinicians in distinguishing HGP from other causes of HMA. Differentiating between HGP and IDA remains a challenge in Vietnam due to the relatively high incidence of both causes, and many medical centers still lack specialized testing for differentiation; it is estimated that about 10-15% of the population in Vietnam carries the thalassemia gene [9]. The results also showed that the IDA group had the highest percentage of pencil cells (44.6%), while tear-drop cells were most prevalent in the HGP group (44.1%); however, the differences were not statistically significant. Most groups had high rates of anisocytosis (> 94%) and annulocytes (> 80%), which can be explained by iron metabolism disorders in IDA and ACD, and globin chain synthesis disorders in HGP, causing abnormalities in RBC size and shape.

Analysis of the ROC curve to determine the risk of HMA compared to the control group showed the cut-off value for serum iron, ferritin, TfS, and UIBC with 47.7 µg/dL (AUC = 0.937, Se = 97.1%, Sp = 75.0%), 112.7 µg/L (AUC = 0.726, Se = 75.0%, Sp = 61.9%), 13.7% (AUC = 0.894, Se = 98.5%, Sp = 69.6%) and 50.0 µmol/L (AUC = 0.313, Se = 60.1%, Sp = 75.0%), respectively (Figure 1). Serum iron and ferritin were commonly ordered tests in the evaluation of anemia, whereas TfS and UIBC were often overlooked; however, studies have demonstrated that TfS and UIBC were also valuable indicators for predicting anemia characterized by microcytic, hypochromic red blood cells. In particular, TfS was calculated based on serum iron and total iron-binding capacity, highlighting its potential for broader clinical application, especially in patients with anemia.

Multivariate logistic regression analysis with iron metabolism assessment indices across HMA cause groups showed that transferrin was an independent predictor of HGP with OR = 0.980, 95%CI = 0.966-0.993, p = 0.004 (Table 5); serum iron, transferrin and TfS were independent predictors of ACD with (OR = 1.089, 95%CI = 1.032-1.150, p = 0.002), (OR = 0.961, 95%CI = 0.946-0.977, $p < 0.001$), (OR = 0.748, 95%CI = 0.625-0.895, p = 0.001) (Table 6). However, no independent predictors were found for IDA (Table 4). In HGP patients, an imbalance in globin chain synthesis causes ineffective hematopoiesis, leading to excessive proerythroblast growth, increased erythroferrone production, and inhibition of hepcidin; furthermore, repeated blood transfusions in transfusion-dependent patients lead to iron accumulation because the body lacks a mechanism to excrete excess iron [10]. In ACD, persistent inflammation induces increased hepatic production of ferritin. At the same time, inflammatory responses lead to the release of multiple cytokines, including IL-6, IL-1, and TNF-α. These cytokines stimulate hepatic synthesis of hepcidin, a key regulator of iron metabolism. Hepcidin suppresses iron absorption by inhibiting ferroportin channels on the basolateral surface of intestinal epithelial cells, thereby reducing iron transport into the circulation. Furthermore, hepcidin limits the mobilization of iron from macrophage stores, which contributes to low serum iron levels and the development of anemia in patients with ACD [11].

In our study, transferrin was an independent predictor in both HGP and ACD (Table 5, Table 6);

this could be explained by the fact that elevated ferritin levels reflect increased iron stores, which suppress hepatic transferrin synthesis, due to these two reasons. Besides, serum iron and TfS were independent predictors of ACD (Table 6). Nevertheless, the optimal cut-off value of transferrin in the two models was similar (Figure 2, Figure 3), which can be explained by the uneven sample size within each cause group. The cut-off value of the TfS index for predicting ACD relative to IDA+HGP was 6.5% (AUC = 0.734, Se = 84.8%, Sp = 58.5%, 95%CI = 0.660-0.807) (Figure 3).

5. CONCLUSION

Patients with hypochromic microcytic anemia presented with diverse clinical symptoms; serum iron, ferritin, TfS, and UIBC levels were predictive of hypochromic microcytic anemia; transferrin was an independent predictor of HGP and ACD, while serum iron and TfS were independent predictors of ACD.

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