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Reticulocyte hemoglobin equivalent as a potential marker for diagnosis of iron deficiency

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Running head: RET-He for diagnosis of iron deficiency

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Abstract

Evaluation of parameters relating to serum ferritin and iron is critically important in the diagnosis of iron deficiency anemia (IDA). The recent development of automated systems for hematology analysis has made it possible to measure reticulocyte hemoglobin equivalent (RET-He), which is thought to reflect iron content in reticulocytes, in the same sample used for complete blood count tests. If RET-He is indeed capable of evaluating iron deficiency (ID), it would be useful for immediate diagnosis of IDA. In the present study, we examined the usefulness of RET-He for diagnosis of ID. Blood samples were obtained from 211 patients. Anemia was defined as hemoglobin (Hb) level of <12 g/dL. Iron-deficiency was defined as serum ferritin level of <12 ng/mL. Patients were classified into four groups: IDA, ID, control, and non-ID with anemia. Patients in the IDA group had significantly lower RET-He levels than those in the control group. RET-He correlated with serum ferritin in the IDA and ID groups. The area under the curve for RET-He was 0.902, indicating that RET-He facilitates the diagnosis of ID with high accuracy. RET-He changed in parallel with changes in Hb during iron administration for 21 IDA patients. Our results indicate that RET-He may be a clinically useful marker for determining ID in the general population.

Keywords: *reticulocyte hemoglobin equivalent (RET-He), iron deficiency anemia, iron, iron deficiency, diagnosis*

INTRODUCTION

Approximately one-third of the world's population suffers from anemia, half of which is due to iron deficiency [1]. Anemia is a significant worldwide health problem with a deleterious influence on mother and child mortality rates, physical performance, and health care [2]. The rate of iron deficiency in the United States has been reported to be 4.5%–18% [3], whereas its prevalence is very high in several other regions, such as Central America (64.7%), South Asia (54.8%), and Andean Latin America (62.3%) [4]. For these reasons, the World Health Organization distributes iron-containing food to prevent iron deficiency in developing countries.

The diagnosis of anemia requires the confirmation of a decrease in hemoglobin (Hb) concentration. In adult men, anemia is diagnosed when Hb concentration is <130 g/L. The cutoff for anemia diagnosis in adult women is a Hb concentration of 120 g/L, whereas this value is slightly lower at 110 g/L for pregnant women [2]. In addition, diagnosis of iron deficiency anemia (IDA) requires information on several additional parameters, such as hematocrit, mean corpuscular volume and Hb, as well as several iron metabolic markers, such as serum iron and serum ferritin levels and total iron binding capacity (TIBC). Among these, serum ferritin, which reflects the total amount of iron the body stores, is a universally available and standardized measurement and is the most effective test to detect iron deficiency. However, false-positive serum ferritin values may be observed because of inflammation, malignancy, or liver disease [5].

With recent technological advances, the Hb content of reticulocytes can be quantified by flow cytometry. Reticulocytes, as immature red blood cells, exist for 1–2 days in the peripheral blood and provide a good index of Hb in newly produced red blood cells and response to iron supplementation [6]. There are several markers for the assessment of Hb content in reticulocytes, including reticulocyte Hb equivalent (RET-He) and reticulocyte Hb content (CHr). RET-He, which can be measured by the latest automated hematology analyzers, is considered to reflect iron content in reticulocytes [7]; it is a direct index of iron availability and reflects cellular availability of iron [8]. CHr and RET-He correlate with iron deficiency and are useful markers of iron deficiency in infants and children, adult blood donors, geriatric patients,

pregnant women, and patients with chronic kidney disease undergoing hemodialysis [9-13]. However, there are only a few studies investigating the diagnosis of iron deficiency in adult patients. In this report, the results of our study evaluating the usefulness of RET-He for iron deficiency diagnosis are presented.

MATERIALS AND METHODS

Patients

This prospective study was approved by the ethics committee of Asahikawa Medical University (authorization numbers 1356, 1679, and 1356-3). Blood samples were obtained from 211 patients treated at Asahikawa Medical University Hospital, Asahikawa-Kosei General Hospital, Engaru-Kosei General Hospital, and Moriyama Hospital between April 2014 and February 2015. Written informed consents were obtained from all patients.

Blood sample collection and measurement of parameters

Blood samples were collected in ethylenediaminetetraacetic acid dipotassium salt (EDTA-2K) tubes and complete blood counts and RET-He levels were determined using an automated hematology analyzer (XN-3000® or XE-5000®, Sysmex, Kobe, Japan). Serum iron (sFe), TIBC, unsaturated iron binding capacity (UIBC), serum ferritin, and biochemical data were collected in plain tubes and measured using an automated chemical analyzer (H-7700 P modular®: Hitachi, Tokyo, Japan). Transferrin saturation (TSAT) was calculated using the following formula: (serum iron/TIBC) × 100. Soluble transferrin receptor (sTfR) was measured by an enzyme-linked immunosorbent assay (Human sTfR Quantikine® IVD® ELISA Kit; R&D Systems) following manufacturer's instruction.

Definition of iron deficiency

In the present study, anemia was defined as an Hb level of <12 g/dL. Iron-deficiency state was defined as serum ferritin level of <12 ng/mL in this study based on previous studies showing serum ferritin below this cutoff value as a highly specific indicator of iron deficiency [14].

Patients were classified into four groups according to their Hb and serum ferritin levels. The IDA group

included patients with both iron deficiency and anemia, defined as serum ferritin and Hb levels of <12 ng/mL and <12 g/dL, respectively. The iron deficiency (ID) group consisted of patients with iron deficiency but no anemia, defined as serum ferritin and Hb levels of <12 ng/mL and ≥12 g/dL, respectively. The control group had neither iron deficiency nor anemia with serum ferritin and Hb levels of ≥12 ng/mL and ≥12 g/dL, respectively. Patients with anemia due to etiologies other than iron deficiency comprised the non-ID with anemia group and had serum ferritin and Hb levels of ≥12 ng/mL and Hb <12 g/dL, respectively.

Determination of changes in RET-He with iron administration during IDA treatment

Among 211 patients included in the present study, 21 IDA patients were treated with oral iron administration and were classified into two groups according to increase or decrease in their Hb levels during treatment. RET-He and serum ferritin levels during treatment were determined for both groups.

Statistical analyses

All data were analyzed using SPSS version 22.0 (IBM). Comparison of groups was performed using the Kruskal–Wallis test. Additionally, Pearson's correlation coefficient and receiver operating characteristic (ROC) plots were used to determine the specificity and sensitivity of RET-He as a marker. *P* values of <0.05 were considered statistically significant for all analyses performed.

RESULTS

Patients' status

A total of 211 patients were enrolled (63 males, 148 females) in the study. The median age was 52.0 years (range, 14–91). The clinical and laboratory parameters of all patients are shown in Table 1. There were 72 (14 males, 58 females), 28 (12 males, 16 females), 67 (23 males, 44 females), and 44 (14 males, 30 females) patients in the IDA, ID, control, and non-ID with anemia groups, respectively. Table 2 shows the clinical and laboratory parameters of patients in each group.

Comparison of RET-He levels according to the iron status at enrollment

Figure 1 shows the RET-He levels in four patient groups at the time of enrollment. The median RET-He levels were 22.3 pg (15.1–35.6 pg), 29.7 pg (19.2–34.9 pg), 34.0 pg (25.9–38.0 pg), and 32.5 pg (19.1–46.3 pg) in the IDA, ID, control, and non-ID with anemia groups, respectively (Table 2). Patients in the IDA group had significantly lower RET-He levels than those in the control group who had neither iron deficiency nor anemia ($p < 0.001$). In addition, the decrease in RET-He levels correlated with the level of iron deficiency (Figure 1; IDA vs ID, $p = 0.016$; ID vs control, $p = 0.033$). Patients in the IDA group had significantly lower RET-He levels than those in the non-ID with anemia group ($p < 0.001$).

Correlation of RET-He with parameters of iron metabolism

Figure 2 shows the relationship of RET-He with sFe, TIBC, and TSAT. The comparison of all patients in the study revealed that RET-He correlated positively with sFe ($r = 0.654$) and TSAT ($r = 0.666$) and correlated negatively with TIBC ($r = -0.617$). As shown in Figure 3, there was no correlation between RET-He and serum ferritin when all patients were included in the analysis ($r = 0.287$); however, analysis of groups according to their iron status revealed a positive correlation between RET-He and serum ferritin in the ID group ($r = 0.604$). Finally, as shown in Figure 4, there was a

negative correlation between RET-He and sTfR, used for iron deficiency diagnosis (r = -0.655).

ROC analysis

Figure 5 shows the ROC analysis that assessed the efficacy of Ret-He in detecting iron deficiency (serum ferritin < 12 ng/mL). The ROC analysis is a method to evaluate usefulness of the test by calculating sensitivity and specificity. The results of this analysis can be shown as the curve in that the sensitivities were plotted against the value of 1-specificity. If the area under the ROC curve (AUC) is almost 1, the accuracy of the test should be considered as excellent. The AUC detecting iron deficiency for RET-He was 0.902, whereas AUC for sFe, TIBC, UIBC, TSAT, and sTfR were 0.889, 0.879, 0.922, 0.917, and 0.821, respectively (data not shown). Using a result of ROC analysis, we calculated the sensitivity of RET-He at the specificity more than 90% to diagnose exactly ID. In that calculation, cutoff value of RET-He was 28.5 pg and the sensitivity was 68%. When we calculated the cutoff of RET-He to diagnose ID at the point with both of high sensitivity and high specificity, the cutoff value should be settled as 30.9 pg. Using this value, sensitivity and specificity should be 92% and 81%, respectively.

Time-course variation in RET-He with iron treatment

Figure 6 shows the time-course variations in serum ferritin and RET-He levels during iron treatment of 21 IDA patients. Among those receiving iron treatments, the Hb levels increased in 14 patients, whereas Hb values decreased or did not change in seven patients during the course of the study. Further assessment revealed that the serum ferritin and RET-He values changed in parallel with changes in Hb levels.

DISCUSSION

Laboratory parameters used for the diagnosis of IDA include sFe, TIBC, TSAT, and serum ferritin. In this study, the cutoff value for serum ferritin for the diagnosis of IDA was 12 ng/mL. Mast *et al.* suggested that the diagnostic accuracy of ferritin could be improved by increasing its cutoff value from 12 μg/L to 30 μg/L; the sensitivity then increased from 25% to 92%, although specificity was unchanged at 98% [15]. Conversely, Ali *et al.* reported that a serum ferritin cutoff value of 12 ng/mL was a highly specific indicator of iron deficiency in a study where iron deficiency was strictly defined based on bone marrow iron stores [14]. Thus, the serum ferritin cutoff level for iron deficiency was set at 12 ng/mL in this study.

RET-He, which was assessed as a potential marker for iron deficiency in this study, has several advantages: rapid measurement in <2 minutes, automatic processing along with complete blood counts, and simple requirement of peripheral blood collected in EDTA tubes. The measurement of RET-He does not require additional reagents [13]. There are numerous other methods to quantify the content in reticulocytes, which includes CHr (Siemens), mean cellular Hb content of reticulocytes (MCHr (Abbott), red blood cell size factor (RSf) (Beckman Coulter), reticulocyte hemoglobin expression (RHE) (Mindray) and reticulocyte hemoglobin cellular content (RHCc) (Horiba) [16]. Quantity of Hb in reticulocytes is a useful marker for patients with specific conditions, such as iron deficient infants and children, adult blood donors, geriatric patients, pregnant women, and patients with chronic kidney disease undergoing hemodialysis [9-13]. In contrast, there are very few studies on iron deficiency in otherwise healthy individuals. Thus, this study included patients of age 15–90 years and provided useful information for general clinical practice.

In this study, 211 adult patients were divided into four groups according to their iron and anemia status, and their RET-He levels were compared. Our results indicated that the patients in the IDA group had significant lower RET-He levels than those in the control group ($p < 0.001$). In addition, the decrease in RET-He depended on the severity

of iron deficiency (Figure 1; IDA vs ID, $p = 0.016$; ID vs control, $p = 0.033$). These results suggested that RET-He reflected the iron state of patients and that it could be a useful marker for the diagnosis of iron deficiency. Furthermore, our data showing that patients in the IDA group had significantly lower RET-He levels than those in the non-ID with anemia group. However, quite variation in RET-He was observed in non-ID with anemia group. Although sufficient data for background was not available, we speculated that this group included various background diseases, for example renal anemia, aplastic anemia (AA), myelodysplastic anemia (MDS) and anemia of chronic disease (ACD) [17]. These diseases cause anemia by different mechanisms, might lead to quite different values in RET-He. For example, AA expected to show normal RET-He value, but some ACD expected to show low RET-He because iron usage should be restricted in inflammation. Future investigation for RET-He especially in non-ID with anemia group with further detail must be needed to elucidate the usefulness of RET-He for differential diagnosis of anemia.

In the present study, to analyze the usefulness of RET-He for determining iron deficiency with high specificity and to simplify classification of the groups, we defined anemia as hemoglobin (Hb) level of less than 120 g/L in both of male and female. However, anemia is also defined as Hb level of <130 g/L in adult men and <120 g/L in adult women in WHO 2011 [2]. When the definition as Hb less than 130 g/L for the diagnosis of anemia in male was used, the number of patients in each group would change: 119 patients in IDA group, 43 patients in ID group, 111 patients in control group and 144 patients in non-ID with anemia group (Supplemental table 1). Even if we used that definition and re-analyze our data, the results was considered to be substantially same; significant difference of RET-He were clearly observed between control and IDA, between control and ID, and between ID and IDA (Supplemental figure 1). This data should also support the usefulness of RET-He for diagnosis of IDA.

We also examined the correlation between RET-He and markers of iron metabolism. Our results indicated that RET-He correlated with several parameters widely used to evaluate the extent of iron deficiency in patients, such as sFe,

TIBC, UIBC, TSAT, and sTfR. While RET-He and serum ferritin did not correlate when all patients in the study were included in the analysis, there was a significant correlation between these two parameters when only patients with iron deficiency were analyzed. One possibility is that the Hb content in reticulocytes might be limited, and RET-He might have reached a maximum point among patients in our study. The ROC analysis for RET-He's ability to diagnose iron deficiency was excellent (AUC, 0.902), which was equal to the AUC values for other iron metabolism parameters, sFe, TIBC, UIBC, TSAT, and sTfR. The cutoff value for RET-He for the diagnosis of ID was 28.4 pg, with a specificity of 91% and a sensitivity of 68%, indicating that its accuracy for ID diagnosis was very similar to those of TIBC, UIBC, TSAT, and sTfR. It would be possible to consider RET-He as an alternative to iron metabolism markers for ID diagnosis if the results were under the cutoff of high specificity.

Analysis of patients receiving oral iron administration revealed that the increase in RET-He mirrored that of Hb in response to treatment and that there was no increase in RET-He values in patients with no change in Hb. Thus, RET-He might be an effective parameter to evaluate response to iron treatment in patients with IDA. Furthermore, RET-He should be considered as a more immediate indicator compared with conventional iron deficiency markers as it reflects the Hb content in reticulocyte immediately after hematopoiesis [6]. In this study, seven IDA patients did not respond to oral iron administration. Unfortunately, the treatment durations differed vastly and the detail information for those patients were not available in the present study, such as the etiology of these non-responders, the dose of iron administration, the compliances of iron administration, and the presence of active bleeding from gastrointestinal tract. Therefore, future studies with predefined treatment durations and dose of iron administration should be needed to evaluate RET-He as a marker both for the diagnosis of IDA and response to iron treatment over a long term.

sTfR is the N-terminal truncated form of transferrin receptor that reflects both the iron deficiency and iron use state in bone marrow; its levels increase in iron deficient states, whereas in patients with excess iron, its levels decreases

[1, 18]. In 2012, a meta-analysis of 10 studies investigating the accuracy of sTfR as a marker for IDA demonstrated its sensitivity and specificity as 86% and 75%, respectively. Compared with other iron metabolism parameters such as serum ferritin, sTfR is also reported not so influenced by inflammation [19]. Thus, the guidelines in the United Kingdom include sTfR as a marker for IDA, although a standardized global cutoff value for sTfR does not exist currently [20]. Our findings in this study clearly demonstrated the association of RET-He with sTfR. This supports that RET-He should be considered as a good marker for ID and IDA as sTfR. There was no significant correlation between RET-He and CRP in the present study (coefficient of correlation 0.02: data not shown), however, if RET-He is not influenced by inflammation should need further study in the patients, especially such as ACD or anemia from cancer.

CONCLUSIONS

In the present study, we evaluated the efficacy of RET-He for IDA diagnosis. Our results indicated that RET-He could be a clinically useful marker for determining iron deficiency in the general population. Therefore, initial measurement of RET-He in patients with anemia prior to other parameters, such as sFe or serum ferritin, might be useful because its assessment is rapid, fully automated, and requires only peripheral blood samples collected to EDTA tubes with no additional reagents. Finally, RET-He might also be used for the evaluation of the efficacy of iron administration.

Conflict of Interest

Yasumichi Toki, Katsuya Ikuta, Mayumi Hatayama, Masayo Yamamoto, Motohiro Shindo, Mikihiro Fujiya, Toshikatsu Okumura (Division of Gastroenterology and Hematology/Oncology, Department of Medicine, Asahikawa Medical University), Masayoshi Kon, Kazuya Sato (Asahikawa-Kosei General Hospital), Motoki Enomoto, Mitsutaka Inoue (Engaru-Kosei General Hospital), Yuko Tada, and Yoko Kikuchi (Moriyama Hospital) received research funding from Sysmex Corporation. This study was also performed in collaboration with Sysmex Corporation. Division of Gastroenterology and Hematology/Oncology, Department of Medicine, Asahikawa Medical University also received collaborative research funding for research work concerning iron metabolism from Chugai Pharmaceutical Co. Ltd., Novartis Pharma K. K., Asahi Kasei Medical Co. Ltd., Shino-Test Corporation and USHIO INC.. Yoshie Kawahara and Noriyasu Niizeki have no conflict of interests to declare.

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Study concept and design: Yasumichi Toki, Katsuya Ikuta, Mikihiro Fujiya, and Toshikatsu Okumura

Laboratory analysis: Yasumichi Toki, Katsuya Ikuta, Yoshie Kawahara, Noriyasu Niizeki, Masayuki Kon, Motoki Enomoto, Satoshi Ito, and Yuko Tada

Statistical analysis and interpretation of data: Yasumichi Toki and Katsuya Ikuta

Obtaining informed consent, sample collection, and analysis of medical data: Yasumichi Toki, Katsuya Ikuta, Mayumi Hatayama, Masayo Yamamoto, Motohiro Shindo, Yoko Kikuchi, Mitsutaka Inoue, and Kazuya Sato

Manuscript preparation: Yasumichi Toki and Katsuya Ikuta

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FIGURE LEGENDS

Figure 1. Reticulocyte hemoglobin equivalent (RET-He) values in patient groups based on their iron status. The horizontal lines represent mean values. *$p < 0.05$

Figure 2. The correlation between reticulocyte hemoglobin equivalent (RET-He) and parameters of iron metabolism. (A) The correlation between RET-He and serum iron (sFe). (B) The correlation between RET-He and total iron binding capacity (TIBC). (C) The correlation between RET-He and transferrin saturation (TSAT). The lines represent approximate straight lines.

Figure 3. The correlation between reticulocyte hemoglobin equivalent (RET-He) and serum ferritin. (A) The correlation between RET-He and serum ferritin in all patients. (B) The correlation between RET-He and serum ferritin in iron deficient patients with a serum ferritin level of <12 ng/mL. The lines represent approximate straight lines.

Figure 4. The correlation between reticulocyte hemoglobin equivalent (RET-He) and soluble transferrin receptor (sTfR). The line represents approximate straight line.

Figure 5. Receiver operating characteristic (ROC) analysis of reticulocyte hemoglobin equivalent (RET-He) in the diagnosis of iron deficiency.

Figure 6. Time-course variation in select parameters of iron metabolism in patients receiving iron treatment. The graphs

in the upper panel show the time-course variations in these parameters of patients whose hemoglobin (Hb) increased with treatment over time. The graphs in the lower panel show the time-course variations in parameters of patients whose Hb either decreased or did not change with treatment over time. (A) and (D) show the changes in Hb levels over time. (B) and (E) show the changes in serum ferritin levels over time. (C) and (F) show the changes in reticulocyte hemoglobin equivalent (RET-He) levels over time.

Supplemental Figure 1. Reticulocyte hemoglobin equivalent (RET-He) values in patient groups based on their iron status. Anemia was diagnosed with hemoglobin level of less than 120 g/L in female but less than 130 g/L in male. The horizontal lines represent mean values. $*p < 0.05$.

Table 1. Clinical and laboratory parameters of 211 patients included in this study

	Reference range	n	Mean (SD)	Median (Range)
Hb (g/dL)	(13.5-17.0)	211	11.6 (2.6)	11.7 (4.1–21.0)
MCV (fL)	(83.0-100.0)	211	85.8 (12.5)	85.9 (55.7–130.8)
Fe (μg/dL)	(80-200)	211	68.8 (52.0)	59.0 (7.9–273.0)
TIBC (μg/dL)	(150-336)	211	370.6 (68.1)	369.0 (193.0–535.0)
UIBC (μg/dL)	(253-365)	211	301.6 (104.0)	306.0 (12.0–519.0)
TSAT (%)		211	20.5 (17.3)	15.7 (1.9–94.2)
Ferritin (ng/ml)	(24.3-166.1)	211	89.4 (273.4)	12.6 (0.0–2598.5)
sTfR (nmol/L)		207	39.5 (30.1)	29.4 (10.8–220.3)
RET-He (pg)		211	29.1 (6.1)	30.4 (15.1–46.3)
TP (g/dL)	(6.0-8.0)	197	7.0 (0.62)	7.0 (4.8–10.6)
Alb (g/dL)	(3.9-4.9)	181	4.2 (0.44)	4.3 (2.5–5.1)
AST (U/L)	(6-40)	201	23.2 (26.2)	19.0 (8.0–372.0)
ALT (U/L)	(6-37)	201	20.3 (34.5)	15.0 (6.0–475.0)
LDH (U/L)	(105-210)	197	190.6 (61.3)	177.0 (107.0–575.0)
γGTP (U/L)	(4-67)	196	29.5 (34.8)	18.0 (5.8–253.0)
BUN (mg/dL)	(6.0-20.0)	200	13.6 (4.4)	13.3 (5.0–29.4)
Cre (mg/dL)	(0.40-1.30)	200	0.68 (0.23)	0.64 (0.20–1.87)
CRP (mg/dL)	(≤0.30)	210	0.19 (0.52)	0.05 (0.00–4.17)
Na (mmol/L)	(135-150)	196	141.2 (2.5)	141.0 (131.0–149.0)
K (mmol/L)	(3.5-5.0)	196	4.2 (0.37)	4.2 (3.2–5.3)
Cl (mmol/L)	(96-110)	195	105.7 (2.4)	106.0 (92.0–112.0)
UA (mg/dL)	(4.0-7.0)	181	4.7 (1.40)	4.4 (2.0–9.3)

Abbreviations: SD, standard deviation; Hb, hemoglobin; MCV, mean corpuscular volume; Fe, serum iron; TIBC, total iron binding capacity; UIBC, unsaturated iron binding capacity; TSAT, transferrin saturation; sTfR, soluble transferrin receptor; RET-He, reticulocyte hemoglobin equivalent; TP, total protein; Alb, albumin; AST, aspartate aminotransferase; ALT, alanine aminotransferase; LDH, lactate dehydrogenase; γGTP, gamma glutamyltranspeptidase; BUN, blood urea nitrogen, Cre, creatinine; CRP, C reactive protein; Na, sodium; K, potassium; Cl, chlorine; UA, uric acid

Table 2. Clinical and laboratory parameters of patients in the four groups

Group		IDA	ID	Control	Non-ID with anemia
Number of patients (Male/female)		72 (14/58)	28 (12/16)	67 (23/44)	44 (14/30)
Age (years)	Mean (SD)	48.7 (18.2)	51.3 (15.0)	55.7 (19.3)	62.5 (19.0)
	Median (Range)	46.0 (14–91)	52.0 (19–71)	62.0 (18–91)	65.0 (16–90)
Hb (g/dL)	Mean (SD)	9.5 (1.8)	13.6 (1.5)	14.0 (1.5)	10.2 (1.4)
	Median (Range)	9.7 (4.1–11.9)	13.3 (12.0–18.5)	13.7 (12.0–21.0)	10.4 (5.6–11.9)
MCV (fL)	Mean (SD)	76.0 (9.8)	82.0 (7.8)	92.4 (5.8)	94.1 (14.4)
	Median (Range)	74.9 (55.7–106.8)	82.5 (67.6–100.2)	91.4 (77.0–108.1)	92.5 (70.9–130.8)
Fe (μg/dL)	Mean (SD)	29.0 (20.7)	51.9 (30.0)	100.7 (44.8)	96.1 (60.6)
	Median (Range)	20.3 (7.9–104.8)	44.2 (18.0–124.0)	94.0 (25.9–234.0)	81.5 (15.0–273.0)
TIBC (μg/dL)	Mean (SD)	423.2 (48.4)	398.7 (50.2)	336.0 (47.1)	319.5 (64.3)
	Median (Range)	426.3 (297.0–535.0)	389.8 (301.0–505.0)	335.0 (246.0–448.0)	312.0 (193.0–475.6)
UIBC (μg/dL)	Mean (SD)	394.2 (52.9)	346.8 (61.2)	235.3 (64.1)	222.5 (103.0)
	Median (Range)	396.0 (253.0–519.0)	340.0 (248.0–478.0)	223.0 (139.0–388.0)	204.5 (12.0–452.0)
TSAT (%)	Mean (SD)	6.9 (4.9)	13.3 (7.9)	30.4 (13.2)	32.4 (21.8)
	Median (Range)	5.3 (1.9–24.1)	11.3 (4.4–33.3)	31.1 (8.4–62.1)	29.1 (3.4–94.2)
Ferritin (ng/ml)	Mean (SD)	5.4 (2.8)	8.0 (2.7)	114.7 (279.1)	240.1 (452.9)
	Median (Range)	5.0 (0.0–11.7)	8.2 (1.7–11.6)	45.4 (12.0–1839.5)	41.2 (12.0–2598.5)
sTfR (nmol/L)	Mean (SD)	57.9 (35.7)	40.1 (24.2)	23.8 (10.8)	33.8 (29.0)
	Median (Range)	47.2 (16.5–220.3)	30.1 (13.6–129.0)	21.5 (11.5–70.1)	25.0 (10.8–158.6)

RET-He (pg)	Mean (SD)	23.4 (4.9)	28.6 (3.6)	33.8 (2.5)	31.9 (5.5)
	Median (Range)	22.3 (15.1–35.6)	29.7 (19.2–34.9)	34.0 (25.9–38.0)	32.5 (19.1–46.3)
TP (g/dL)	Mean (SD)	7.1 (0.69)	7.1 (0.40)	7.1 (0.47)	6.7 (0.73)
	Median (Range)	7.1 (5.7–10.6)	7.0 (6.2–7.7)	7.1 (6.2–8.3)	6.8 (4.8–8.4)
Alb (g/dL)	Mean (SD)	4.1 (0.47)	4.3 (0.30)	4.4 (0.30)	4.0 (0.53)
	Median (Range)	4.2 (2.5–4.8)	4.3 (3.7–5.1)	4.4 (3.6–5.1)	4.1 (2.6–4.9)
AST (U/L)	Mean (SD)	19.6 (9.4)	21.2 (7.3)	23.1 (9.1)	30.1 (53.2)
	Median (Range)	17.0 (8.0–71.0)	19.0 (13.0–52.0)	21.0 (11.0–56.0)	21.5 (11.0–372.0)
ALT (U/L)	Mean (SD)	13.8 (5.4)	22.0 (18.7)	20.4 (13.0)	29.1 (70.0)
	Median (Range)	12.0 (6.0–30.0)	16.0 (7.0–100.0)	16.0 (6.0–59.0)	15.5 (6.0–475.0)
LDH (U/L)	Mean (SD)	170.2 (33.6)	180.7 (36.5)	196.2 (69.6)	219.6 (79.6)
	Median (Range)	164.0 (119.0–267.0)	178.0 (110.0–257.0)	183.0 (116.0–575.0)	208.5 (107.0–479.0)
γGTP (U/L)	Mean (SD)	19.1 (17.5)	31.4 (29.0)	33.9 (35.5)	37.7 (50.6)
	Median (Range)	14.0 (7.0–125.0)	20.2 (11.0 – 121.0)	19.0 (5.8–194.0)	19.0 (6.0–253.0)
BUN (mg/dL)	Mean (SD)	12.8 (4.3)	13.9 (3.9)	13.0 (4.1)	15.5 (4.9)
	Median (Range)	12.3 (6.1–29.0)	14.0 (6.7–26.2)	12.9 (5.8–21.7)	14.3 (5.0–29.4)
Cre (mg/dL)	Mean (SD)	0.62 (0.19)	0.69 (0.20)	0.67 (0.18)	0.78 (0.31)
	Median (Range)	0.59 (0.20–1.48)	0.66 (0.44–1.34)	0.64 (0.37–1.27)	0.71 (0.38–1.87)
CRP (mg/dL)	Mean (SD)	0.07 (0.13)	0.10 (0.15)	0.22 (0.55)	0.38 (0.87)
	Median (Range)	0.03 (0.00–0.75)	0.05 (0.00–0.66)	0.05 (0.00–4.04)	0.05 (0.00–4.17)
Na (mmol/L)	Mean (SD)	140.3 (2.3)	141.3 (2.7)	142.0 (2.5)	141.3 (2.1)

	Median (Range)	140.0 (134.0–148.0)	141.0 (136.0–149.0)	142.0 (131.0–147.0)	141.0 (137.0–145.0)
K (mmol/L)	Mean (SD)	4.2 (0.41)	4.2 (0.35)	4.2 (0.33)	4.2 (0.39)
	Median (Range)	4.3 (3.2–5.3)	4.2 (3.5–4.9)	4.2 (3.3–5.1)	4.2 (3.3–4.9)
Cl (mmol/L)	Mean (SD)	105.7 (2.1)	105.2 (3.5)	105.5 (2.4)	106.0 (2.1)
	Median (Range)	106.0 (99.0–111.0)	105.0 (92.0–110.0)	106.0 (96.0–111.0)	106.0 (101.0–112.0)
UA (mg/dL)	Mean (SD)	4.1 (1.48)	4.8 (1.25)	5.1 (1.28)	4.6 (1.32)
	Median (Range)	4.0 (2.0–8.5)	4.3 (2.8–7.4)	4.9 (3.1–8.0)	4.5 (2.4–9.3)

Abbreviations: IDA, iron deficiency anemia; ID, iron deficiency; SD, standard deviation; Hb, hemoglobin; MCV, mean corpuscular volume; Fe, serum iron; TIBC, total iron binding capacity; UIBC, unsaturated iron binding capacity; TSAT, transferrin saturation; sTfR, soluble transferrin receptor; RET-He, reticulocyte hemoglobin equivalent; TP, total protein; Alb, albumin; AST, aspartate aminotransferase; ALT, alanine aminotransferase; LDH, lactate dehydrogenase; γGTP, gamma glutamyltranspeptidase; BUN, blood urea nitrogen, Cre, creatinine; CRP, C reactive protein; Na, sodium; K, potassium; Cl, chlorine; UA, uric acid

Table 1. Clinical and laboratory parameters of 211 patients included in this study

	Reference range	n	Mean (SD)	Median (Range)
Hb (g/dL)	(13.5-17.0)	211	11.6 (2.6)	11.7 (4.1–21.0)
MCV (fL)	(83.0-100.0)	211	85.8 (12.5)	85.9 (55.7–130.8)
Fe (μg/dL)	(80-200)	211	68.8 (52.0)	59.0 (7.9–273.0)
TIBC (μg/dL)	(150-336)	211	370.6 (68.1)	369.0 (193.0–535.0)
UIBC (μg/dL)	(253-365)	211	301.6 (104.0)	306.0 (12.0–519.0)
TSAT (%)		211	20.5 (17.3)	15.7 (1.9–94.2)
Ferritin (ng/ml)	(24.3-166.1)	211	89.4 (273.4)	12.6 (0.0–2598.5)
sTfR (nmol/L)		207	39.5 (30.1)	29.4 (10.8–220.3)
RET-He (pg)		211	29.1 (6.1)	30.4 (15.1–46.3)
TP (g/dL)	(6.0-8.0)	197	7.0 (0.62)	7.0 (4.8–10.6)
Alb (g/dL)	(3.9-4.9)	181	4.2 (0.44)	4.3 (2.5–5.1)
AST (U/L)	(6-40)	201	23.2 (26.2)	19.0 (8.0–372.0)
ALT (U/L)	(6-37)	201	20.3 (34.5)	15.0 (6.0–475.0)
LDH (U/L)	(105-210)	197	190.6 (61.3)	177.0 (107.0–575.0)
γGTP (U/L)	(4-67)	196	29.5 (34.8)	18.0 (5.8–253.0)
BUN (mg/dL)	(6.0-20.0)	200	13.6 (4.4)	13.3 (5.0–29.4)
Cre (mg/dL)	(0.40-1.30)	200	0.68 (0.23)	0.64 (0.20–1.87)
CRP (mg/dL)	(≤0.30)	210	0.19 (0.52)	0.05 (0.00–4.17)
Na (mmol/L)	(135-150)	196	141.2 (2.5)	141.0 (131.0–149.0)
K (mmol/L)	(3.5-5.0)	196	4.2 (0.37)	4.2 (3.2–5.3)
Cl (mmol/L)	(96-110)	195	105.7 (2.4)	106.0 (92.0–112.0)
UA (mg/dL)	(4.0-7.0)	181	4.7 (1.40)	4.4 (2.0–9.3)

Abbreviations: SD, standard deviation; Hb, hemoglobin; MCV, mean corpuscular volume; Fe, serum iron; TIBC, total iron binding capacity; UIBC, unsaturated iron binding capacity; TSAT, transferrin saturation; sTfR, soluble transferrin receptor; RET-He, reticulocyte hemoglobin equivalent; TP, total protein; Alb, albumin; AST, aspartate aminotransferase; ALT, alanine aminotransferase; LDH, lactate dehydrogenase; γGTP, gamma glutamyltranspeptidase; BUN, blood urea nitrogen, Cre, creatinine; CRP, C reactive protein; Na, sodium; K, potassium; Cl, chlorine; UA, uric acid

Table 2. Clinical and laboratory parameters of patients in the four groups

Group		IDA	ID	Control	Non-ID with anemia
Number of patients (Male/female)		75 (17/58)	25 (9/16)	62 (18/44)	49 (19/30)
Age (years)	Mean (SD)	49.1 (18.0)	50.4 (15.1)	54.2 (19.1)	63.7 (18.3)
	Median (Range)	47.0 (14–91)	50.0 (19–70)	58.5 (18–91)	69.0 (16–90)
Hb (g/dL)	Mean (SD)	9.6 (1.9)	13.7(1.5)	14.1 (1.5)	10.4 (1.5)
	Median (Range)	10.0 (4.1–12.7)	13.4(12.0–18.5)	13.9 (12.0–21.0)	10.5 (5.6–12.8)
MCV (fL)	Mean (SD)	75.8 (9.6)	83.2 (7.0)	92.2 (5.9)	94.1 (13.5)
	Median (Range)	75.8 (55.7–106.8)	83.5 (70.2-100.2)	91.0 (77.0–108.1)	93.0 (70.9–130.8)
Fe (μg/dL)	Mean (SD)	29.1 (20.3)	54.2 (29.9)	102.7 (45.0)	94.0 (57.7)
	Median (Range)	20.3 (7.9–104.8)	44.7 (18.0–124.0)	94.0 (31.0–234.0)	82.0 (15.0–273.0)
TIBC (μg/dL)	Mean (SD)	420.6 (49.4)	403.3 (48.1)	339.2 (47.1)	317.1 (60.8)
	Median (Range)	422.7 (297.0–535.0)	404.1 (303.1–505.0)	339.7 (246.0–448.0)	307.0 (193.0–475.6)
UIBC (μg/dL)	Mean (SD)	391.5 (53.5)	349.2 (62.2)	236.5 (65.4)	222.3 (97.0)
	Median (Range)	393.0 (253.0–519.0)	338.0 (248.0–478.0)	225.5 (139.0–388.0)	208.0 (12.0-452.0)
TSAT (%)	Mean (SD)	7.90 (4.9)	13.8 (8.0)	30.8(13.3)	32.9 (27.7)
	Median (Range)	5.3 (1.9–24.1)	12.0 (4.4–33.3)	31.0 (8.4–62.0)	25.0 (10.8–158.6)
Ferritin (ng/ml)	Mean (SD)	5.4 (2.8)	8.2 (2.7)	119.5 (287.3)	221.2 (428.1)
	Median (Range)	5.1 (0.0–11.7)	8.9 (1.7–11.6)	41.4 (12.0–1839.5)	44.8 (12.0–2598.5)
sTfR (nmol/L)	Mean (SD)	58.5 (35.9)	36.1 (16.3)	23.9 (10.9)	32.9 (27.7)
	Median (Range)	47.9 (16.4–220.3)	29.6 (13.6–79.5)	21.5 (11.4–70.1)	25.0 (10.8–158.6)

RET-He (pg)	Mean (SD) Median (Range)	23.4 (4.9) 22.4 (15.1–35.6)	29.1 (2.9) 29.8 (22.5–34.9)	33.7 (2.6) 34.0 (25.9–38.0)	32.1 (5.2) 32.6 (19.1–46.3)
TP (g/dL)	Mean (SD) Median (Range)	7.1 (0.67) 7.1 (5.7–10.6)	7.1 (0.41) 7.0 (6.2–7.7)	7.1 (0.46) 7.1 (6.2–8.3)	6.8 (0.73) 6.9 (4.8–8.4)
Alb (g/dL)	Mean (SD) Median (Range)	4.1 (0.46) 4.2 (2.5–4.8)	4.3 (0.31) 4.3 (3.7–5.1)	4.4 (0.30) 4.4 (3.6–5.1)	4.1 (0.52) 4.2 (2.6–4.9)
AST (U/L)	Mean (SD) Median (Range)	19.5 (9.2) 17.5 (8.0–71.0)	21.7 (7.3) 20.5 (14.0–52.0)	23.6 (9.2) 21.5 (11.0–56.0)	28.9 (50.0) 21.0 (11.0-372.0)
ALT (U/L)	Mean (SD) Median (Range)	13.7 (5.3) 12.0 (6.0–30.0)	23.4 (19.0) 17.0 (11.0–100.0)	21.0 (13.2) 17.0 (6.0–59.0)	27.4 (65.8) 15.0 (6.0–475.0)
LDH (U/L)	Mean (SD) Median (Range)	170.6 (33.3) 164.5 (119.0–267.0)	181.0 (36.3) 177.0 (110.0–257.0)	197.9 (71.4) 183.5 (116.0–575.0)	215.3 (76.0) 200.0 (107.0–479.0)
γGTP (U/L)	Mean (SD) Median (Range)	19.3 (17.0) 14.0 (7.0–125.0)	32.6 (30.0) 20.0 (11.0 – 121.0)	34.7 (36.3) 18.0 (5.8–194.0)	36.4 (48.0) 20.0 (6.0–253.0)
BUN (mg/dL)	Mean (SD) Median (Range)	12.9 (4.2) 12.4 (6.1–29.0)	13.7 (4.0) 13.4 (6.7–26.2)	12.8 (4.1) 12.9 (5.8–21.7)	15.5 (4.7) 14.5 (5.0–29.4)
Cre (mg/dL)	Mean (SD) Median (Range)	0.63 (0.19) 0.60 (0.20–1.48)	0.70 (0.21) 0.66 (0.44–1.34)	0.66 (0.18) 0.63 (0.37–1.27)	0.79 (0.29) 0.72 (0.38–1.87)
CRP (mg/dL)	Mean (SD) Median (Range)	0.07 (0.12) 0.03 (0.00–0.75)	0.11 (0.15) 0.05 (0.00–0.66)	0.15 (0.28) 0.05 (0.00–1.72)	0.45 (0.97) 0.05 (0.00–4.17)
Na (mmol/L)	Mean (SD)	140.3 (2.2)	141.5 (2.8)	142.0 (2.6)	141.4 (2.1)

	Median (Range)	140.0 (134.0–148.0)	141.0 (136.0–149.0)	142.0 (131.0–147.0)	141.0 (137.0–145.0)
K (mmol/L)	Mean (SD)	4.2 (0.40)	4.2 (0.36)	4.2 (0.33)	4.2 (0.37)
	Median (Range)	4.3 (3.2–5.3)	4.2 (3.5–4.9)	4.2 (3.3–5.1)	4.2 (3.3–4.9)
Cl (mmol/L)	Mean (SD)	105.7 (2.1)	105.3 (3.6)	105.5 (2.4)	106.0 (2.0)
	Median (Range)	106.0 (99.0–111.0)	105.0 (92.0–110.0)	105.5 (96.0–111.0)	106.0 (101.0–112.0)
UA (mg/dL)	Mean (SD)	4.1 (1.45)	4.89 (1.23)	5.1 (1.26)	4.8 (1.34)
	Median (Range)	4.0 (2.0–8.5)	4.37 (2.8–7.4)	4.8 (3.1–8.0)	4.7 (2.4–9.3)

Abbreviations: IDA, iron deficiency anemia; ID, iron deficiency; SD, standard deviation; Hb, hemoglobin; MCV, mean corpuscular volume; Fe, serum iron; TIBC, total iron binding capacity; UIBC, unsaturated iron binding capacity; TSAT, transferrin saturation; sTfR, soluble transferrin receptor; RET-He, reticulocyte hemoglobin equivalent; TP, total protein; Alb, albumin; AST, aspartate aminotransferase; ALT, alanine aminotransferase; LDH, lactate dehydrogenase; γGTP, gamma glutamyltranspeptidase; BUN, blood urea nitrogen, Cre, creatinine; CRP, C reactive protein; Na, sodium; K, potassium; Cl, chlorine; UA, uric acid