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Original Article

Percentage of hypochromic red cells: a better predictive marker than conventional hematological parameters in the diagnosis of latent iron deficiency in women with menometrorrhagia

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Abstract: Patients with Latent iron deficiency (LID) may have inconspicuous signs, so they rarely go to seek medical attention. Besides that, LID is neglected by many physicians. Moreover, conventional hematological indicators do not meet the clinical need for early diagnosis of LID. Therefore, this study was aimed to verify the clinical usefulness of percentage of hypochromic red cells (% HYPO) as an early predictor of LID in women with menometrorrhagia. One hundred forty-two female outpatients in gynecologic clinic (age range, 21-51 years) with menometrorrhagia (bleeding >7 days in duration with an average blood loss >80 ml) were selected in China. These subjects were divided into 3 groups: a LID group (or iron deficient without anemia), an iron deficiency anemic group (IDA), and a control group without iron deficiency. Iron deficiency = LID + IDA. There were 40 subjects with LID, 63 subjects with IDA, and 39 control subjects. We measured the % HYPO, red blood cell (RBC) count, hemoglobin (Hb), hematocrit (HCT), mean corpuscular volume (MCV), red blood cell distribution width (RDW), serum ferritin (SF), serum iron (SI), transferrin saturation (TS), and total iron binding capacity (TIBC). Receiver operating characteristics analysis was performed to assess % HYPO and other conventional hematological parameters in identifying LID. Of the 142 subjects, 40 (28.17%) were LID. % HYPO was 0.5 (0.3, 0.9) in healthy controls, 5.6 (2.3, 8.9) in subjects with LID, and 28.4 (13.6, 53.5) in subjects with IDA. Receiver operator characteristic (ROC) curves demonstrated that the area under the curve (AUC) for % HYPO (0.901) was significantly greater than that for RDW (0.768), MCV (0.759), Hb (0.721), in the diagnosis of LID. The results showed that % HYPO was a better predictor than conventional hematological parameters in the diagnosis of LID in women with menometrorrhagia. We recommend that % HYPO be integrated into the protocols for the diagnosis of LID in women with menometrorrhagia.

Keywords: Percentage of hypochromic red cells, latent iron deficiency, iron deficiency, iron deficiency anemia, menometrorrhagia

Introduction

Iron deficiency (ID) is the most common nutritional deficiency in the world [1]. Latent iron deficiency (LID), also called iron-deficient erythropoiesis [2] is a medical condition in that there is evidence of iron deficiency without anemia (normal hemoglobin).

Initially, menometrorrhagia, which occurs in up to 24% of women ages 40-50 years [3], places women at higher risks for iron negative balance due to menstrual blood loss. In addition, many women patients with LID may have inconspicuous "non-hematological" signs or symptoms: fatigue, hair loss, reduced immune response, reduced performance, reduction in cognitive function, poor work productivity, and poor attention and memory [4-7], so they rarely seek medical attention. Furthermore, some physicians may diagnose ID only based on the values of RBC, Hb and the other conventional hematological indicators, which have low sensitivity and specificity, so LID may be easily misdiagnosed. What's more, early identification and treatment of LID are imperative for preventing the patients from developing IDA and the deleterious effects of the disease. Therefore, the goal of this study was to investigate the clinical application and effectiveness of % HYPO, a new

Table 1. Comparisons of Hematological and Biochemical Indicators between the control group and the LID group

	Control	LID	t or z	P
Patient number	39	40		
Gender	Female	Female		
Age (years)	40.0±6.0	40.5±6.0	-0.351	0.727*
BMI (kg/m^2)	21.3±1.9	20.9±1.3	1.088	0.281*
SBP (mmHg)	118.9±7.4	116.9±8.7	1.140	0.258*
DBP (mmHg)	75.3±6.2	76.6±5.1	-0.979	0.330*
% HYPO (%)	0.5 (0.3, 0.9)	5.6 (2.3, 8.9)	-6.139	<0.001#,a
RBC (*10^{12}/L)	4.38±0.29	4.61±0.51	-2.446	0.017*,a
Hb (g/L)	131.4±10.0	123.6±10.2	3.439	0.001*,a
HCT (%)	39.1±2.7	38.1±2.8	1.703	0.093*
MCV (fL)	89.4±5.2	82.8±6.9	4.793	<0.001*,a
RDW (%)	13.2 (12.7, 13.6)	14.2 (13.4, 15.7)	-4.104	<0.001#,a
SF (μg /L)	48.0 (26.1, 63.0)	6.3 (3.6, 10.2)	-7.649	<0.001#,a
SI (μmol/L)	16.0 (12.5, 16.5)	10.7 (7.8, 16.0)	-2.850	0.004#,a
TIBC (μmol/L)	71.4 (67.1, 87.1)	82.0 (76.3, 86.7)	-2.457	0.014#,a
TS (%)	23.0 (16.0, 25.5)	15.0 (9.6, 20.0)	-3.407	0.001#,a

*Student's t-test was used in comparing statistical significance of Age, BMI, SBP, DBP, RBC, Hb, HCT and MCV (normally distributed variables) between two groups (a: P-value of <0.05 was considered statistically significant). #The Mann-Whitney U Tests were used to compare the % HYPO, RDW, SF, SI, TIBC, and TS (non-normally distributed variables) between two groups (a: P-value of <0.05 was considered statistically significant).

RBC-extended parameter, which has not been studied for the detection of LID, compared to traditional hematological parameters in the early diagnosis of LID in women with menometrorrhagia.

Materials and methods

Subjects

142 female outpatients with menometrorrhagia, defined as persistent bleeding lasting longer than 7 days with an average blood loss of more than 80 ml, were enrolled between December 2011 and December 2012 at the gynecologic clinics of Air Force General Hospital in China based on their medical history. LID was defined as a ferritin level of <14 μg/L and a hemoglobin level ≥110 g/L, and IDA was defined as a ferritin level of <14 μg/L and a hemoglobin level of <110 g/L. Exclusion criteria included inflammation, cancer, pregnancy, disorders of hemostasis, and other organic diseases. Of the 142 subjects enrolled in this study, 40 had LID (age 40.5±6.0 years), 63 had IDA (38.5±7.2 years) and 39 were healthy controls (age 40.0±6.0 years). Informed consents of all participants were obtained prior to participation in this research. Experiments involving human subjects in this study have been in accord with the Helsinki Declaration of 1975.

Laboratory assays

Fasting venous blood samples were collected in EDTA vacutainer tubes and analyzed for the following hematological parameters: % HYPO, RBC, Hb, HCT, MCV, and RDW. Analyses were performed within 2 hours after collection on a Siemens ADVIA 120 Hematology Analyzer. Subsequently, the blood samples were centrifuged, the plasma was separated, and the plasma samples were stored at -20°C. Analyses of SF, SI, TIBC, and TS were performed within 1 month on a Hitachi 7180 Biochemistry Automatic Analyzer.

Statistical analysis

All calculations were performed using SPSS version 19.0. Depending on the data distribution (as assessed by the Kolmogorov-Smirnov test), parametric and nonparametric tests were applied. Continuous normally distributed variables were expressed as mean values ± standard deviations. Non-normally distributed variables were expressed as the median value and interquartile range (IQR). For normally distributed variables, Student's t-tests were used to compare the differences between two groups. For non-normally distributed variables, the Mann-Whitney U test was used to compare the differences between two groups. Student's t-tests were used to compare the differences in the values including Age, body mass index (BMI), Systolic Blood Pressure (SBP), Diastolic Blood Pressure (DBP), RBC, Hb, HCT, and MCV between the control group and the LID group (**Table 1**). The Mann-Whitney U test was used to compare the differences in the values including % HYPO, RDW, SF, SI, TIBC, and TS between the control group and the LID group (**Table 1**). Student's t-tests were used to compare the dif-

Table 2. Comparisons of Hematological and Biochemical Indicators between the LID group and the IDA group

	LID	IDA	t or z	P
Patient number	40	63		
Gender	Female	Female		
Age (years)	40.5±6.0	38.5±7.2	-1.438	0.154*
BMI (kg/m^2)	20.9±1.3	20.4±1.2	1.830	0.070*
SBP (mmHg)	116.9±8.7	113.3±9.2	1.967	0.052*
DBP (mmHg)	76.6±5.1	72.1±6.1	4.001	<0.001*,a
% HYPO (%)	5.6 (2.3, 8.9)	28.4 (13.6, 53.5)	-6.242	<0.001#,a
RBC (*10^{12}/L)	4.61±0.51	4.00±0.50	5.941	<0.001*,a
Hb (g/L)	123.6±10.2	91.4±14.3	13.305	<0.001*,a
HCT (%)	38.1±2.8	30.1±3.4	12.258	<0.001*,a
MCV (fL)	82.8±6.9	75.8±8.6	4.323	<0.001*,a
RDW (%)	14.2 (13.4, 15.7)	16.8 (15.3, 18.3)	-5.216	<0.001#,a
SF (μg /L)	6.3 (3.6, 10.2)	6.4 (3.4, 9.8)	-0.873	0.383#
SI (μmol/L)	10.7 (7.8, 16.0)	11.8 (7.2, 18.3)	-0.098	0.922#
TIBC (μmol/L)	82.0 (76.3, 86.7)	82.7 (73.2, 90.3)	-0.284	0.776#
TS (%)	15.0 (9.6, 20.0)	15.0 (10.0, 21.0)	-0.230	0.818#

*Student's t-test was used in comparing statistical significance of Age, BMI, SBP, DBP, RBC, Hb, HCT and MCV (normally distributed variables) between two groups (a: P-value of <0.05 was considered statistically significant). #The Mann-Whitney U Tests were used to compare the % HYPO, RDW, SF, SI, TIBC, and TS (non-normally distributed variables) between two groups (a: P-value of <0.05 was considered statistically significant).

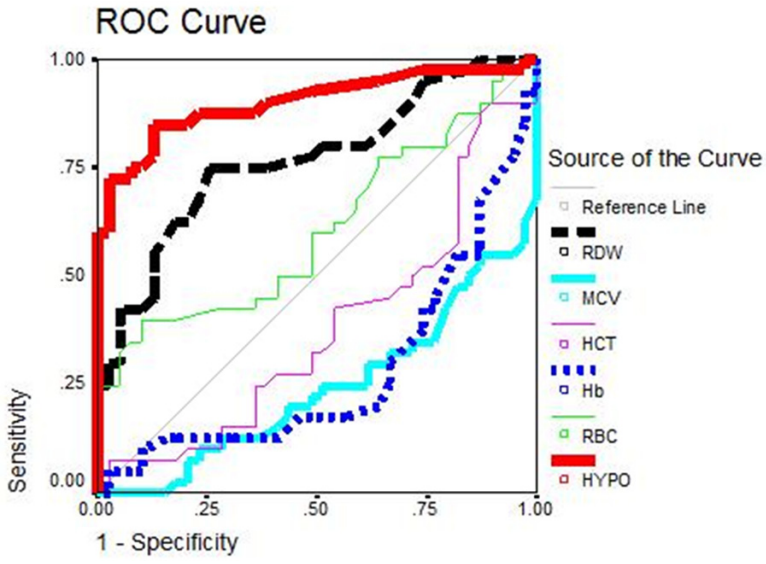

Figure 1. Receiver Operating Characteristic curves for % HYPO and conventional hematological indicators in the diagnosis of LID.

ferences in the values including Age, BMI, SBP, DBP, RBC, Hb, HCT, and MCV between the LID group and the IDA group (**Table 2**).

The Mann-Whitney U test was used to compare the differences in the values including % HYPO, RDW, SF, SI, TIBC, and TS between the LID group and the IDA group (**Table 2**). A receiver operating characteristic (ROC) curve, including the area under the curve (AUC), was used to evaluate the diagnostic performance of % HYPO and other traditional RBC parameters in the diagnosis of LID. A two-tailed P-value of <0.05 was considered statistically significant.

Results

The prevalence rate (PR) of LID in women with menometrorrhagia

In this study, the prevalence rate (PR) of LID (40/142) was 28.17%.

The comparisons of hematological and biochemical indicators between the control group and the LID group

The comparisons of hematological and biochemical indicators in the control group and the LID group were shown (**Table 1**). There was no significant difference in age, BMI, SBP, and DBP between the two groups (P>0.05, respectively). The median value of % HYPO was significantly higher in LID group than in the control group (P<0.001). The LID group had significantly lower Hb, MCV, SF, SI, and TS values compared to the control group (P<0.01, respectively). The LID group had higher RBC, RDW and TIBC values than the control group (P<0.05, respectively). There was no significant difference between the two groups with respect to HCT (P = 0.093).

Table 3. Receiver Operating Characteristic curves for % HYPO and conventional hematological indicators in the diagnosis of LID

	AUC	Cutoff value	PPV	NPV	P
% HYPO (%)	0.901	1.55	0.872	0.850	<0.001
RDW (%)	0.768	13.55	0.750	0.744	<0.001
MCV (fL)	0.759	81.0	0.397	0.063	<0.001
Hb (g/L)	0.721	113.5	0.493	0.252	0.001
HCT (%)	0.624	35.2	0.507	0.500	0.057
RBC (*10^{12}/L)	0.609	3.915	0.520	0.750	0.096

The comparisons of hematological and biochemical indicators between the LID group and the IDA group

The comparisons of hematological and biochemical indicators in the LID group and the IDA group were shown (**Table 2**). There was no significant difference in age, BMI, and SBP between the two groups (P>0.05, respectively). The mean DBP levels were significantly higher in the LID group than in the IDA group (P<0.001). The median values of % HYPO and RDW were significantly lower in LID group than in the IDA group (P<0.001, respectively). The LID group had significantly higher RBC, Hb, HCT, and MCV values compared to the IDA group (P<0.001, respectively). There were no significant differences between the two groups with respect to SF, SI, TIBC, and TS (P>0.05, respectively).

ROC curve analysis used to evaluate the reliability of various indicators in diagnosing LID

ROC curve analysis was performed to evaluate the reliability of various indicators in diagnosing LID (**Figure 1**). The results of ROC analysis were summarized (**Table 3**). AUC for % HYPO (P<0.001), RDW (P<0.001), MCV (P<0.001), Hb (P = 0.001), HCT (P = 0.057), and RBC (P = 0.096) was 0.901, 0.768, 0.759 (1-0.241), 0.721 (1-0.279), 0.624 (1-0.376), and 0.609, respectively. Because AUC showed that the P values of RBC and HCT were greater than 0.05, RBC and HCT were not useful in diagnosing LID. ROC curves showed that % HYPO had a better predictive ability than RDW, MCV, Hb in diagnosing LID in women with menometrorrhagia. The best cut-off point for % HYPO was 1.55 corresponding to a sensitivity of 85.0%, a specificity of 87.2%, a positive predictive value (PPV) of 0.872, and a negative predictive value (NPV) of 0.85 for LID.

Discussion

It has been reported that the prevalence rate (PR) of LID was 15.1% according to women's health exams [8]. In this study, the prevalence rate of LID in women with menometrorrhagia (28.17%) was significantly higher than that in women's health exams. The patients and doctors should pay more attention to LID in women with menometrorrhagia.

The comparisons of hematological indicators between the control group and the LID group showed that there was no significant difference between the two groups with respect to HCT. Although there were significant differences in the values of RBC and Hb between the 2 groups, the values of RBC and Hb in the 2 groups were within normal range.

ROC curves showed further evidence that RBC and HCT were not useful in diagnosing LID (AUC showed that the P values of RBC and HCT were greater than 0.05 in the diagnosis of LID). Although AUC showed that the P value of Hb was less than 0.05 in the diagnosis of LID, Hb should not be used for the diagnosis of LID because the values of Hb in the LID group were still within the normal lab reference range, resulting in a relative insensitivity (i.e., due to the marked overlap in values between normal and iron deficiency populations). Therefore, RBC, Hb and HCT may be not valuable in the early diagnosis of LID.

There were significant differences in biochemical indicators (SF, SI, TS and TIBC) between the control group and LID group (P<0.05, respectively). There were no significant differences in biochemical indicators between the LID group and IDA group (P>0.05, respectively). This study proved biochemical indicators were useful in early diagnosis of LID. The Biochemical indicators are commonly used in the detection of iron deficiency. But biochemical indicators have limitations. Serum iron exhibits diurnal variations, and may transiently reach reference values after the ingestion of meat or oral iron supplements. Ferritin is regarded as the best standard iron store biochemical indicator. However, the concentration of ferritin rises in the presence of inflammatory response, and thus the diagnosis of LID can be particularly challenging in patients with acute or chronic inflammatory conditions. Transferrin is also an acute-phase

reactant. The concentrations of TIBC may be affected by certain medications.

The Prussian blue stain of bone marrow aspirate is regarded as the gold standard test for iron deficiency. But because it is invasive, painful, and costly, its clinical application is limited.

Recently, there has been increased interest in the use of new hematological parameter, % HYPO, which was available on modern hematology analyzers. % HYPO was obtained with the Semenzi ADVIA, by the manufacturer's definition, based on the optical cell-by-cell hemoglobin by using a high-angle scatter, determined on the previously sphered erythrocytes [9].

In this study, the values of % HYPO in the LID group (5.6 (2.3, 8.9)) was significantly higher than in the control group (0.5 (0.3, 0.9)) and significantly lower than in the IDA group (28.4 (13.6, 53.5)) ($P<0.001$, respectively). AUC provided further evidences that % HYPO had a better predictive ability than other conventional hematological parameters (RBC, Hb, MCV, HCT, and RDW) in diagnosing LID. Conventional hematological parameters have their limitations in detecting LID because red blood cells have a long lifespan of approximately 4 months, and they can not provide real-time information.

% HYPO may meet the clinical need because the direct consequence of an imbalance between the erythroid marrow iron requirements and the actual supply is a reduction of red cell hemoglobin content. This reduction causes an increase in hypochromic mature red cells and provides useful information about individual cell characteristics [10, 11]. Moreover, % HYPO may have the bonus benefit of being relatively uninfluenced by inflammatory responses [12]. Furthermore, % HYPO can be measured as a by-product of routine complete blood cell count within the same EDTA blood sample, and this would result in a significant reduction in the amount of blood needed for clinical diagnosis. Finally, all of the results of % HYPO and complete blood cells can be obtained in a short time by modern automatic hematology analyzers. Therefore, the measurement of % HYPO by the Siemens hematological analyzer is sensitive, specific, fast, efficient, and practical. For the above reasons, % HYPO may surpass other conventional hematological parameters in the diagnosis of LID.

However, a major obstacle to the use of % HYPO is its reduced availability. % HYPO is obtained using the Siemens ADVIA and is patented. % HYPO-He, obtained by Sysmex, can provide clinicians with information equivalent to % HYPO [9]. Low hemoglobin density (LHD%), obtained by Beckman-Coulter, has a good correlation with % HYPO. LHD% could be a reliable parameter for the study of iron metabolism status [13]. These new laboratory biomarkers of hypochromia were defined by different automatic hematological analyzer manufacturers.

Other studies showed that IDA and anemia of chronic disease (ACD) patients were efficiently differentiated using % $HYPO_m$ [14] and hypochromic erythrocytes (%), a reliable marker for recognizing iron-restricted erythropoiesis and predicting responsiveness to erythropoietin in anemic patients with myeloma and lymphoma [15].

Our research suggested that because the prevalence rate of LID in women with menometrorrhagia (28.17%) was very high, both the patients and their doctors should pay more attention to LID. Our data showed that % HYPO may be a better predictor of LID compared to conventional hematological parameters in women with menometrorrhagia. The measurement of % HYPO is sensitive, specific, fast, efficient, and practical. We conclude the % HYPO may be valuable for the early prediction of LID. Therefore, we recommend that % HYPO be integrated into the protocol in the diagnosis of LID in women with menometrorrhagia. Further studies are warranted to determine whether % HYPO should be the preferred screening tool for the early detection of LID in larger, unselected populations of patients.

Disclosure of conflict of interest

None.

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