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Research Article

Evaluation of Red Cell Distribution Width and Platelet Distribution Width as a Predictor of Iron Stores; A Comparative Cross-Sectional Study

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ABSTRACT

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Keywords: Iron deficiency anemia, Iron stores, Ferritin, PDW, RDW



This article is an open access article distributed under the

terms and conditions of the Creative Commons Attribution (CC BY) license http://creativecommons.org/licenses/by/4.0/ **Background:** Iron deficiency (ID) is a global problem that affects women more prevalently, causing vast health consequences. Screening by hemoglobin and serum ferritin is currently applied for ID. Recently, interest in complete blood count parameters (CBC) has emerged for its close relationship with body iron stores.

Objectives: to examine whether hematological indices of CBC [namely red cell distribution width-coefficient of variation (RDW-CV) and platelets distribution width-coefficient of variation (PDW-CV)] can serve as a predictor for ID in Iraqi women.

Subjects and Methods: A cross-sectional study at the National Center of Hematology recruited 200 women into a study group: 100 anemic women and 100 healthy controls. Their demographics, hematological indices, and iron indicators (serum ferritin, iron, total iron binding capacity (TIBC), and transferrin saturation) were collected.

Results: The RDW-CV and platelet counts were statistically higher in the IDA cases, while PDW was lower. RDW had significant correlation to serum ferritin (r=-0.2, P=0.04), while PDW was significantly linked to serum ferritin (0.22, P=0.03) TIBC (r=-0.24, P=0.02) and TSP (r=-0.23, P=0.02). RDW had the highest positive and negative predictive values, with 84 and 97 %, respectively, followed by platelet counts, which scored 72 and 78 %, respectively. RDW-CV cutoff value scored the highest sensitivity, 97%, and specificity, 82%, in discriminating ID cases from healthy controls with an area under the curve of 0.98 and P<0.001.

Conclusions: RDW showed significant correlations to iron indicators with strong discriminating power and high positive predictive value. RDW accessibility and affordability make them recommendable markers in earlier screening for ID.

Introduction

Globally, iron deficiency (ID) is the most prevalent deficiency of a single nutrient in industrialized as well as developing nations (1) In contrast to men, females exhibit a reduced circulating blood volume, diminished red cell count, and diminished hemoglobin mass, all of which contribute to an elevated susceptibility to anemia among females, especially among pregnant and menstruating women (2). Erythropoiesis is frequently maintained in ID, which ultimately results in iron deficiency anemia (IDA) in the advanced stages (3)

Therefore, a significant portion of the burden of ID in women will go unnoticed if it is presumed that adequate iron stores are present in the absence of anemia. Having said that, ID is significantly more prevalent than IDA (4). Women diagnosed with ID experience compromised physical capabilities, such as muscular fatigue, cognitive impairments including short-term memory loss and diminished attention, and ultimately, a decline in their quality of life (QOL) (5).

IDA is a composite diagnosis determined by ferritin and hemoglobin (Hb) concentrations. Presently, serum ferritin is thought to be the most useful metric for assessing ID (6). Nonetheless, subsequent testing has examined other complete blood count (CBC) hematological markers. They have the advantage of being available, reasonably priced, and, according to a recent trend, maybe more indicative of a number of ID symptoms, as they indicate cellular iron deficiency in erythroblasts before anemia (7). Red blood cell distribution width (RDW), including RDW-coefficient of variation (RDW-CV), is a widely accessible and easy hematological metric that indicates the extent of erythrocyte size diversity (8). It is frequently employed to investigate differential diagnoses for anemia, specifically IDA (9). Reactive thrombocytosis was reported in IDA. Nevertheless, the fundamental mechanism remains poorly understood. Certain scholars have postulated that the observed changes in platelet counts and their indices platelet distribution width (PDW) and mean platelet volume (MPV) are the result of platelet morphological alterations (10) (11). This study aims to examine the relation between hematological parameters (namely RDW-CV and PDW-CV) as predictors for iron stores among Iraqi women.

Subjects and Methods

Across-sectional research was conducted at the National Center of Hematology/Mustansiriyah University in Baghdad/Iraq from January 2023 to August 2023. The study included anemic women attending our center during the reference period who agreed to participate after we explained the study's aim and objective. The study protocol was approved by the ethical committee of Mustansiriyah University No. 247, Issued in January 2023. The Declaration of Helsinki was followed. The study group was anemic women (aged >18 years) diagnosed according to the WHO criteria for anemia in non-pregnant women where a Hb concentration of less than 12 g/l and ferritin less than 20 μ g/l g (12)was used to confirm the diagnosis.

An exclusion was made to cases with a body mass index (BMI) above 30 kg/m², women with a history of acute or chronic infection or hemoglobinopathy, those on drugs like steroids or aspirin, and cases with metabolic diseases and liver and kidney disease were all omitted. An aged and BMI-matched group of healthy controls were also recruited. A total of 200 women were analyzed for the study, where 100/200 were anemic, and 100/200 were healthy controls.

The demographic criteria, including age, body weight, and height (for the calculation of BMI), were taken. Additionally, a sample of venous blood was taken and divided into two: The first tube was a three ml blood for assessing ferritin (ng/ml), iron (μ g/dL), total iron binding capacity (TIBC (μ g/dl), and transferrin saturation (%). Ferritin was measured by fluorescence Immunoassay (ichroma II). Roche Cobas 111 Chemistry analyzer calculated serum iron and TIBC. Transferrin saturation was calculated as the ratio of serum iron to TIBC, then multiplied by 100 to create a percent.

The second tube was a two-ml blood with [EDTA] anticoagulant for CBC test analyzed by a fully automatic NORMA Icon 3 hematology analyzer. From the CBC obtained, we recorded red blood cell counts (RBC), hemoglobin (Hb), red blood cells distribution width-coefficient of variation (RDW-CV), platelet counts (PLT), mean platelets volume, platelets distribution width-coefficient of variation (PDW-CV), and white blood cell counts (WBC).

The study sample size was calculated according to the formulae(13) (14).

Sample size: Based on the cross-sectional study sampling formula used to estimate the

Sample size =[$(Z_{(1-\alpha/2)}^{2*}SD^{2}]/d^{2}$

Z is the value of standard variate at five %, which is 1.96. level of significant

*D is the accuracy (2.25) while SD is the standard deviation taken from the earlier research (1.20).

Eighty-nine was the minimum sample size needed, doubled by two due to the utilization of two groups (178) and the addition of 10% non-response. There were 200 total participants in this study—100 IDA patients and 100 healthy controls.

Statistical analysis:

Data collected for this research were checked and entered through the software program Statistical Package for Social Sciences (SPSS) version 26. Descriptive statistics was done by presenting data by frequency and percentage for categorical variables. For numerical continuous variables, mean and standard deviation (SD) were used. Inferential statistics was done using different statistical tests. Independent samples t-test was used to assess the mean differences between two independent groups of the sample. Pearson Correlation was used to assess the significance, strength, and direction of the correlation between quantitative variables. By using the Receiver Operator Characteristic Curve (ROC), the cutoff points for the hematological test between anemic patients and controls were detected. The positive and negative predictive values (PPV and NPV) were calculated to test the performance of study parameters in screening for anemia. We considered that a P-value less than 0.05 is significant.

Results

A comparative study recruited 200 women into 100 IDA cases and 100 controls. There was no statistical significance regarding the age (28.69 \pm 12.35 *vs*. 30.15 \pm 10.44 years; P=0.38) and body mass index (23.64 \pm 5.24 vs. 23.45 \pm 3.42 kg/m²; P=0.76) in the IDA cases *vs*. healthy controls respectively.

Table 1. Shows that the Hb, iron, ferritin, and transferrin saturation were significantly higher among the healthy controls with P < 0.001. In contrast, TIBC was significantly higher in the IDA group. As for hematological parameters, WBC counts were statistically insignificant among the participants. The RDW-CV and platelet counts were statistically higher in the IDA cases. PDW-CV, MPV, and RBC counts were meaningfully higher in healthy controls.

Table 2. Pearson correlation was done; it highlighted a statistically significant correlation between RDW vs. serum ferritin (r=-0.2, P=0.04), while PDW was significantly linked to serum ferritin (0.22, P=0.03) TIBC (r=-0.24, P=0.02) and TSP (r-0.23, P=0.02). The rest of the parameters had statistically insignificant links.

BMI: Body mass index, Hb: Hemoglobin, TIBC: Total iron binding capacity

RDW-CV: Red blood cells distribution width-coefficient of variation, MPV: Mean platelets volume, PLT: Platelets, PDW: Platelets distribution width, WBC: White blood cell counts, RBC: Red blood cell* indicate statistical significant <0.05

RDW-CV: Red blood cells distribution width-coefficient of variation, MPV:Mean platelets volume, PDW: Platelets distribution width, WBC: White blood cell counts, TSP: Transferrin saturation (%).* indicates statistically significant <0.05

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P=0.03) TIBC (r=-0.24, P=0.02) and TSP (r-0.23, P=0.02). The rest of the parameters had statistically insignificant links.

 Table 2: Pearson correlation between hematological parameters vs. iron indicators in the study participant

Variable		Iron	Ferritin	TIBC	TSP
RDW-CV (%)	R	-0.07	-0.20	-0.11	-0.045
	P-value	0.50	0.04*	0.26	0.66
PDW-CV (%)	R	0.12	0.22	-0.24	0.23
	P-value	0.22	0.03*	0.02*	0.02*
MPV (fL)	R	-0.12	-0.13	- 0.008	0.06
	P-value	0.25	0.22	0.94	0.53
Platelet counts	R	-0.15	0.005	0.06	-0.17
	P-value	0.14	0.96	0.54	0.09



Figure 1: The ROC curve for the study parameters that discriminated IDA cases vs. controls

For the sake of clarification, we added Table 3 to illustrate the cutoff value, sensitivity, specificity, 95% confidence interval, PPV, and NPP alongside the respective P-value of hematological indices shown in Fig.1. The highest PPV and NPV were for RDW, with 84 and 97 percent, respectively, followed by platelet counts, which scored 72 and 78 percent, respectively. MPV and PDW-CV showed 63, 62%, and 90,66% for the PPV and NPV, respectively.

Discussion

This study analyzed hematological indices in Iraqi women as possible iron deficiency (ID) predictors. The control group showed significantly higher Hb, iron, ferritin, and transferrin saturation vs. anemic cases, which was in accordance with earlier works(2) (7) As for the hematological indices, they were significantly higher among controls, including PDW-CV, MPV, and RBC counts. The ID can have clinical implications on a woman's health even in the

participants		. nennatorogreui		ig uie stady
Variable	The Groups are (n=100) for each	Mean	Standard Deviation	P-value
$\text{Ub}(\alpha/\text{I})$	IDA	0 00	1.59	

Table 1: The basic biochemical and hematological criteria among the study

	(n=100) for each		Deviation	
Hb (g/L)	IDA	8.89	1.58	
	Healthy control	13.40	0.94	<0.001*
Iron (µg/dL),	IDA	16.58	8.88	
	Healthy control	74.36	19.71	<0.001*
Ferritin	IDA	9.68	4.90	
(112/1111),	Healthy control	50.30	17.88	<0.001*
TIBC (µg/dl)	IDA	428.88	100.15	
	Healthy control	309.28	37.55	<0.001*
Transferrin	IDA	4.05	2.65	
saturation (%)	Healthy control	23.72	5.70	<0.001*
RDW-CV (%)	IDA	18.50	3.14	
	Healthy control	13.36	0.81	<0.001*
PDW-CV (%)	IDA	47.40	4.07	
	Healthy control	50.10	5.51	<0.001*
MPV (fL)	IDA	7.77	1.43	
	Healthy control	8.20	1.07	0.016*
PLT (counts)	IDA	362.41	100.25	
	Healthy control	263.99	57.11	<0.001*
WBC (counts)	IDA	7.057	1.46	
	Healthy control	7.14	1.14	0.639
RBC (counts)	IDA	4.35	0.50	
	Healthy control	4.70	0.36	<0.001*

In Figure 1, The ROC was constructed for RDW-CV, PDW-CV, MPV, and platelets counts to determine the parameters' cutoff value associated with best sensitivity (SN)and specificity (SP) in screening for ID. RDW scored the highest SN of 97% and SP of 82%, P<0.001, followed by platelet counts with SN of 81% and SP of 69%, P<0.001. All tested indices showed a statistically significant P-value of <0.001 and an area under the curve of (0.98,0.81,0.07, and 0.63), respectively.

presence of a normal Hb, hematocrit, RBC counts, MCV, and MCHC value (6)

Table 3: illustrates the cutoff value of hematological indices that distinguished IDA cases from healthy controls alongside their area under the curve, 95% confidence interval, sensitivity, specificity, positive predictive value, negative predictive value, and respective P-value.

Parameters	AUC	95 % CI	Cutoff point	SN	SP	PPV	NPV	P- value
RDW-CV (%)	0.98	0.962- 1.00	≥ 14.25	97%	82%	84%	97%	< 0.001*
PDW-CV (%)	0.67	0.589- 0.741	≤ 50.35	73%	52%	60%	66%	< 0.001*
MPV (fL)	0.63	0.553- 0.714	≤ 7.75	61%	63%	63%	62%	0.001*
Platelet's count	0.81	0.745- 0.868	285.50	81%	69%	72%	78%	< 0.001*

RDW-CV: Red blood cells distribution width-coefficient of variation, MPV: Mean platelets volume; PDW: Platelet distribution width; CI: confident interval, AUC: Area under the curve, SN: sensitivity, SP: specificity; PPV: positive predictive value; NPP: negative predictive value.* indicate statistical significance <0.05

Various indicators observed on a peripheral blood smear can provide useful insights about the underlying cause of anemia. For instance, anisocytosis may occur before the development of microcytic-hypochromic changes in the early stages of IDA. It is important to note that these changes may be consistent with a diagnosis of ID, but they are not definitively indicative(15)

The analysis showed higher RDW measurement in ID cases, which is in line with earlier works that agreed on this sign as 1st indicator of ID.

Variability in the red blood cell (RBC) size is a reflection of anisocytosis, which is manifested in CBC before the IDA actually occurs(16) (17)

IDA only occurs after 3 stages. The 1^{st} when serum ferritin is depleted in the stores; both anisocytosis and microcytosis are evidence. Later in 2^{nd} stage, the hemoglobin will decrease with a compensatory increase in erythropoietic activity. Finally, the 3^{rd} stage is where low ferritin, hemoglobin, and reduced MCHC finally occur (18)

The current study linked RDW-CV to serum ferritin and TSP, while PDW was correlated to ferritin, TIBC, and TSP. Furthermore, RDW was a good predictor of serum iron compared to PDW, which served as a predictor of serum ferritin.

A cross-sectional prospective study by Figueroa-Mujica et al. examined the correlation of serum ferritin with RDW and PDW in women throughout all the pregnancy trimesters in high vs. low attitude settings. In line with our analysis, their results confirmed that both parameters were significantly linked to ferritin irrespective of the pregnancy trimester and the attitude where the women lived(19)

Tiwari et al. study discussed a significant association between RDW-CV and serum ferritin in a sample of Indian pregnant women in their 2^{nd} and 3^{rd} trimesters. They recommended that RDW screen for IDA(20)

Those results aligned with earlier research on American women(21) Another study by Rivera et al. examined CBC parameters diagnosing deficient iron stores in Filipino women; their analysis found that those parameters were unsatisfactory in discriminating deficient stores. However, the ability to confirm IDA was satisfactory. They confirmed that the RBC count showed the highest association and AUC for IDA women(21)

Akkermans *et al.*'s study tested the role of RDW and platelet counts in screening for iron stores among children less than three years old. In accordance with our results, RDW was negatively linked to ferritin yet it showed low specificity and sensitivity to predict deficient iron stores and IDA. Platelet counts had no role. The authors did not recommend RDW as a primary predictor for this age group (22)

In an Indian study that involved IDA children under the age of five years, RDW was found to be a reliable predictor of the IDA and its severity; it increased as the severity of anemia increased especially among severe-moderate cases(14)

Our data showed increased platelet counts among cases with deficient iron stores, which was in line with published literature(23)

The low iron stimulates megakaryocytes in the bone marrow by multiple mechanisms:

Higher influx of cells into the megakaryocytic compartment with higher efflux rates, shorter maturation of the megakaryocyte, and a compensatory mechanism for inhibited erythropoiesis(24)

Kadikoylu et al. study linked PDW with iron indices in IDA females. PDW was not found to be correlated to iron indices; they only correlated to mean platelets volume and mean corpuscular volume, respectively(25)

We have to acknowledge the inconsistency in some earlier works, which may be attributed to factors that can affect the CBC parameters, especially among those with anemia, including duration of anemia, severity, sampling time, patient's gender, age, and the presence of iron supplements(21).

A Dutch study discussed RDW reliability in anemic children under the age of three of RDW, even in the presence of an infection(22)

A recent Saudi study discussed that RDW measurement was unaffected by age, unlike platelet counts, which showed higher values in younger ages(26)

Another Iraqi study confirmed the reliability of RDW with respect to BMI in a sample of pregnant women(27)

The RDW-CV was also used in microcytic anemic cases to discriminate between IDA and other causes of anemia, like the thalassemia trait(28)

A metanalytic study by Hoffmann *et al.* discussed that none of the tested CBC indices are 100 percent sensitive or specific, but they may serve as an adjuvant tool to reach the final diagnosis(29)

Study strength

CBC parameters are readily, routinely, and cheaply obtained; their changes are the earliest signs of anemia as they precede other biomarkers(7)

The close relationship between iron indicators and CBC-obtained red cell indices with high sensitivity, specificity, and a significant AUC, as seen in ROC analysis, makes using them in practice as a surrogate measure of iron deficiency and iron deficiency appealing not to mention their good predictive power and independence of the age and BMI extremities which adds more credibility to their use.

Serum ferritin is the gold standard for iron stores(6)

yet its levels are confounded by many factors, including inflammation, infections, metabolic and endocrine syndromes, liver diseases, and medications (30,31). Therefore, the application of additional diagnostic indicators for depleted iron stores and IDA is needed.

Study limitations and recommendations

The confounders of serum ferritin were not addressed here; a single-center study limited globalization of the current results (32) and the sensitivity and specificity were not high for all tested parameters, although their statistical significance was high P<0.001. We suggest a combination of those CBC parameters in predicting the iron stores to improve their predictive value.

Most earlier studies involved pregnant rather than non-pregnant women, which warrants further work in that area. Being crosssectional is a limitation, too, where the cause and effect cannot be ascertained. We recommend a long prospective study to see the impact of these changes. It will be interesting to know the role of hematological parameters in the diagnosis, treatment, and prognosis of affected cases.

Conclusion

The accessibility, availability, and significance of RDW in screening for iron indices with good sensitivity and specificity made it recommendable for application in practice.

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