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The Utility of Hypochromic Markers in the Detection of Latent Iron Deficiency in Women in their First Trimester of Pregnancy

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Abstract

Background and Objectives: While iron deficiency anemia is a documented common problem in pregnancy, there is limited information on latent iron deficiency. Accordingly, we aimed to determine the frequency of the latter in the first trimester of pregnancy and assess the utility of new hypochromic markers in screening for it.

Methods: In this cross-sectional study, we recruited 100 pregnant women in their first trimester attending the Maternity Hospital in the period between January and March 2023. Inclusion criteria included hemoglobin >11.0 g/dl, and no iron supplements. Serum ferritin was used to classify patients into latent iron deficiency or iron replete state. While a hematology analyzer was used to determine the new hypochromic markers: namely %Hypo-He, Ret-He, % Hyper-He, RBC-He.

Results: The mean age of enrollees was 29.3 (SD 5.5) years and included 38% with latent iron deficiency (hemoglobin > 11 g/dl and S. ferritin ≤ 30 ng/ml). The latter group had significantly lower hemoglobin (P=0.03), and %Hypo-He (P<0.001) than iron replete women. While neither MCV, MCH, reticulocyte counts, Ret-He, or RBC-He were different. Furthermore, %Hypo-He was significantly negatively correlated with serum ferritin (P<0.001) and had a fair discriminating power between latent iron deficiency and iron replete status.

Conclusion: High prevalence of latent iron deficiency was documented among pregnant women in their first trimester; Furthermore, %Hypo-He was a better red cell index to screen for it. Because of its high frequency and anticipated impact on fetal and maternal health, routine screening for latent iron deficiency in pregnancy may be warranted.

Key words: Latent Iron deficiency, Pregnancy, Red cell indices.

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Introduction

Worldwide, iron deficiency is common in women during their reproductive years, especially during pregnancy [1] and it is not a problem restricted to developing countries, but is also prevalent in developed countries.[2] The higher frequency of iron deficiency among women is the consequence of menstruation and pregnancies, where the physiological adaptations are insufficient to meet the increased requirements and nutritional need for iron is not met.[3] The development of iron deficiency starts with depletion of iron stores, and this is followed by iron-deficient erythropoiesis, and culminates with development of overt iron deficiency anemia. Latent iron deficiency (LID) is a term used in an individual who has normal hemoglobin but reduced storage iron as reflected by reduced serum ferritin. A normal hemoglobin in the first trimester of pregnancy is defined by the WHO as hemoglobin > 11 g/dl [4], while the optimal cut-off serum ferritin for considering LID was set at ≤30 ng/ml by most authorities.[5-7] As iron deficiency progresses, red cell indices like mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) decrease, while red cell distribution width (RDW) increases, and these indices are routinely generated by traditional hematology analyzers. More advanced instruments introduced new red cell parameters, which may be more informative. One of these parameters is % hypochromic red cells (% Hypo-He) which measures the percentage of hypochromic red cells with MCH < 17 pg. Other parameters include hemoglobin content of mature RBC (RBC-He) and hemoglobin content of reticulocytes (Ret-He); the latter serves as an indicator of iron availability for red cell production in the last four days (lifespan of reticulocyte in the bone marrow and circulation).[8] Latent iron deficiency is condition which has often been overlooked and only limited number of studies addressed its clinical significance, particularly in pregnancy. On the other hand, several studies looked at the utility of new red cell parameters in the diagnosis of iron deficiency (including LID)[5] its differentiation from thalassemia and anemia of chronic disorders, and in assessing response to iron therapy though to our knowledge none tackled this issue in our country.[9] Accordingly, in the current study we aimed to determine the prevalence of latent iron deficiency in pregnant women in their first trimester and to determine the utility of the new hypochromic red cell indices in the diagnosis of this deficiency.

Materials and methods

This is a cross-sectional study which included 100 pregnant women (age between 18-40 years) in their first trimester of pregnancy (first 12 weeks) visiting the maternity hospital in Duhok-Iraq, in the period between January and March 2023. For inclusion in the study, the hemoglobin concentration should be more than 11 g/dL, and the patient should not be on any iron supplementation at the time of enrollment. Each patient had five ml of venous blood aspirated, which was divided between a 3ml in plain tube (clotted sample), and 2ml anticoagulated in EDTA. The latter sample was used to do complete blood counts (Swelab, Boule Medical AB, Sweden), and when Hb >11.0 mg/dl, demographic data were collected including age, duration of pregnancy (by ultrasound, pregnancy test, or missed periods). Thereafter, the patient was asked about number of abortions/pregnancies/live births, and time since last delivery. The serum sample was used to determine S. ferritin concentration (Cobas c501, Roche Diagnostics, HITACHI, Tokyo, Japan). Based on S. ferritin concentration the enrollees were classified into either latent iron deficiency (LID) when their serum ferritin was ≤ 30 ng/ml; or iron replete individuals when their serum ferritin was in excess of 30 ng/ml.[5] Furthermore, all

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EDTA samples who had a hemoglobin > 11 gm/dl were run through a Sysmex XE 5000 instrument (Sysmex Corporation, Kobe, Japan), which is a fully automated hematology analyzer that provides complete blood cell and leukocyte differential counts.[10] It utilizes flow fluorescence cytometry technology, the analyzer generates in addition to traditional red cell indices, absolute and relative reticulocyte counts, new RBC parameters including: % hypochromic RBC (%Hypo-He) representing the percentage of red cell with MCH <17 pg, % Hyperchromatic RBC (%Hyper-He) representing percentage of RBC with MCH >49 pg, Hemoglobin content of mature RBC (RBC-He), and reticulocyte hemoglobin content (Ret-He).[8] The study was approved by the ethics committee at the Kurdistan Higher Council of Medical Specialties and an informed consent was obtained from all enrolled women. For statistical analysis an SPSS software (IBM corp., SPSS v22, USA) was used for most statistical assessments. A median and interquartile range or mean and standard deviation (SD) were used to represent continuous variables as appropriate. Man, Whitney U test and Spearman correlation (both non-parametric) were used as required. MedCalc Software V22.005 (Belgium) was used for Receiver Operating Characteristic (ROC) analysis and Area Under Curve (AUC) assessment to determine the discriminative ability of significantly identified red cell indices. P < 0.05 was considered significant.

Results

The pregnant women enrolled had a mean age (SD) of 29.3 (5.5) years. Their mean (SD) gestational age at the time of enrollment was 8.3 (2.6) weeks. They included 20 primigravida, while the remaining 80 had a median number of previous pregnancies of 3 (range 1-10). Forty-four women had a history of previous abortions (median 1 [range 1-4]). Thirty-eight patients (38%) were categorized as Latent Iron Deficiency (LID) based on Ferritin ≤ 30ng/ml, while the remaining 62 were regarded as iron replete. The LID subgroup included 19 women whose serum ferritin was ≤20 ng/ml, and 11 whose S. ferritin was ≤ 15 ng/ml. Table (1) compares between the various clinical and hematological features in LID and iron replete women. Women with LID had significantly lower hemoglobin and hematocrit than those who were iron replete (P values of 0.030, and 0.049 respectively). MCV, MCH, and RDW were not significantly different. The only other significantly different hematological parameter was the % hypochromic RBC (% Hypo-He) which was significantly higher in LID compared with Iron replete women (P<0.001). Figure (1) shows the distribution of % Hypo-He in LID and iron replete women. Ret-He, on the other hand, was not significantly different. The median time since the last delivery was lower in LID compared iron replete women, though this just failed to reach significance (P=0.051). More severe iron depletion as reflected by lower ferritin cut-offs (≤20 and ≤15 ng/ml) were both associated with significantly lower hemoglobin and higher %Hypo-He (P<0.001 and 0.001 respectively). Furthermore, the ≤15 ng/ml cut off was also associated with reduced Ret-He (p=0.034). Moreover, it was found that the area under curve for % Hypo-He increased progressively from the ≤30 to the ≤ 15 ng/ml. Table (2), indicating increasing discriminating power. The best sensitivity and specificity combination (81.8% and 76.4% respectively) was obtained with ≤15 ng/ml S. Ferritin categorization at a cut-off point of 0.30 of % Hypo-He.

Table (1): A comparison between various parameters in latent Iron deficiency (LID) compared to Iron replete women in the current study.

	Median (Interquartile Range		
Parameter	LID	Iron replete	P
Age (years)	28 (24-31)	29.5 (26-36)	0.059
Gestational age (weeks)	8(6-10)	9 (7-10)	0.177
Number of pregnancies	3(2-4)	3 (2-4)	0.775
Number of abortions	0 (0-1)	0 (0-1)	0.862
Time since last birth (years)*	2.0 (1.0-4)	3.0 (2.0-5.0)	0.051
Hemoglobin (g/dL)	12.2 (11.6-13.0)	12.6 (12.3-13.3)	0.030
RBC (x 10^{12}/L)	4.3 (4.18-4.62)	4.47 (4.24-4.71)	0.176
Hct (%)	36.5(35-39.5)	37.9 (36.5-39.7)	0.046
MCV (fL)	84.0 (82.0-88.5)	85.6 (83.0-88.2)	0.629
MCH (pg)	28.5 (26.4-29.3)	28.6 (27.7-29.7)	0.632
RDW (%)	13.3 (12.9-14.3)	13.2 (12.7-13.8)	0.186
RBC-He (pg)	29.2 (7.5-29.9)	29.5 (28.2-30.6)	0.192
Hypo-He (%)	0.4 (0.2-0.8)	0.2 (0.18-0.3)	<0.001
Hyper-He (%)	0.7 (0.5-0.8)	0.7 (0.6-0.8)	0.395
Reticulocytes (x 10^{12}/L)	0.061 (0.048-0.079)	0.068 (0.054-0.102)	0.081
Ret-He (pg)	31.3 (29.2-31.8)	31.6 (29.9-33.0)	0.873

*In 80 women who had previous pregnancies.

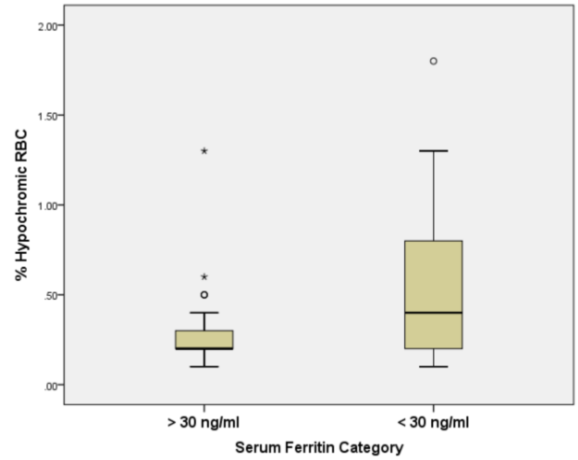

Figure (1): A boxplot showing the distribution of % hypochromic cells in Latent iron deficiency and in iron replete women in the current study.

Table (2): Area under curve (AUC) for % hypochromic RBC using three different cut-off points for S. Ferritin, their significance, cut-off values and sensitivity and specificity for each.

Ferritin cut-off	AUC (SE)	95 % CI*	P value	Cut-off % Hypo-He	Sensitivity (%)	Specificity (%)
≤ 15 ng/ml	0.789 (070)	0.652 – 0.926	<0.001	>0.30	81.8	76.4
≤ 20 ng/ml	0.753 (0.061)	0.633-0.873	<0.001	>0.30	63.2	77.8
≤ 30ng/ml	0.709 (0.057)	0.597-0.821	<0.001	>0.30	55.26	85.5

*CI: confidence intervals

Ferritin had a significantly positive correlation with hemoglobin (r=0.253; P=0.011) and negative one with % hypochromic RBC (r=-0.423; P<0.001) Figure (2). Furthermore, there were

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significant positive correlations between % Hypo-He and RDW (r=0.450; P<0.001), and negative ones with Ret-He (r=-0.515; P< 0.001), hemoglobin (r=-0.321; P=0.001), MCV, MCH and reticulocyte absolute count (P<0.001, 0.001 and 0.009 respectively).

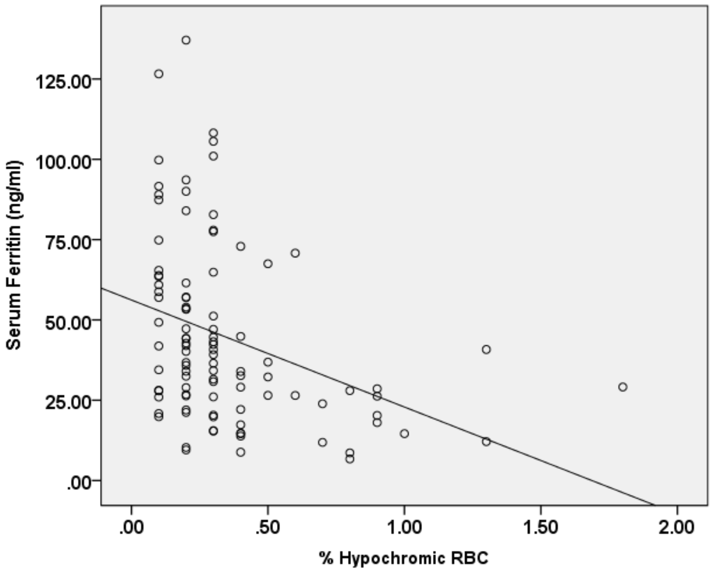

Figure (2): Scatterplot showing the correlation between Serum Ferritin and % hypochromic red cells (spearman coefficient= - 0.423; P<0.001).

Discussion

Iron sufficiency throughout pregnancy is essential for fetal and maternal health and identification of iron deficiency whether latent or frank and its management are of paramount important during pregnancy.[11] Although it is well documented that overt iron deficiency anemia is quite frequent in pregnancy, data on latent iron deficiency (iron deficiency without anemia) are scarce, though some believe it to be at least twice as frequent as overt iron deficiency anemia.[3,12] We chose the cut-off point of 30 ng/ml for diagnosis of LID and not cut-off of 15 ng/ml as proposed by the WHO for iron deficient stores in pregnant women in their first trimester of pregnancy though the latter cut off point is highly specific, yet it lacks sensitivity, and the higher cut-off point of 30 ng/ml may be more reflective of functional iron deficiency as it better correlates with marrow storage iron and has a high sensitivity (92%) and specificity (98%).[5,7,11] Based on the latter cut-off point 38% of enrolled women in the current study were categorized as LID, and this observation is consistent to a great extent with a report of Urrechaga et al (2016) that reported LID at 38.8% of their non-anemia Spanish premenopausal women.[13] Much higher prevalence rate of LID were reported in Asian developing countries with rates as high as 66.25% among Sri Lankan women in their first trimester.[14] Higher rates of iron deficiency were reported in second and third trimesters.[15] Women with LID in the current study tended to be younger, and had lower median time since their last pregnancy than iron replete women (though both just failed to reach significance), this is consistent with previous studies which found that young age and shorter times since last pregnancies were predictors of iron deficiency in pregnancy.[16] The current study showed that women with LID had lower hemoglobin, and hematocrit than iron replete women, while neither the MCV, RDW or MCH were significantly different.[14] This in contrast to one earlier study which found MCV and MCH were lower and another one finding that RDW was significantly higher in LID pregnant Sri Lankan ladies.[17] Of the new red cell indices, % Hypo-He was significantly higher in LID compared to iron replete women in the current study, and S. Ferritin was significantly negatively correlated with % Hypo-He. Interestingly, it was found that as the degree of iron store depletion increases, other parameters will become more relevant with Ret-He becoming significant when the Ferritin cut-off point of ≤15 ng/ml was chosen, so did the RBC-He and MCH, while the significance of RDW approached but did not reach significance. Furthermore, it was

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noted that % Hypo-He had increasing area under curve (increasing discriminating power) with decreasing iron stores, and good sensitivity and specificity at a cut-off point of 0.35 when a ferritin ≤ 15 ng/ml was chosen. This is to a great extent consistent with the literature, with several authors identifying % Hypo-He as a better predicator of iron deficiency than Ret- He [18-20] ,though others studies suggested better or equal predictive results of Ret-He compared to % Hypo He.[21-23] The importance of identifying and treating latent Iron deficiency in the first trimester, stems from the observations that iron deficient pregnant women (whether they are anemic or not) are more likely to develop pre and postnatal iron deficiency, low birth weight babies, intra-uterine growth retardation and prematurity.[15,24] Iron deficiency anemia and LID in pregnant women have also been linked to mental illness, impaired neurocognitive functions, and behavioral disturbances in their babies that may present later in their lives.[25,26] Accordingly, it is now recommended that LID should be treated once it has been identified in early pregnancy.[11,16]

Conclusions

The current study revealed that nearly two fifths of pregnant women in their first trimester have latent iron deficiency, and that the % Hypo-He is a superior predictor for latent iron deficiency than other red cell indices. In view of the possible consequences of unmanaged LID, the need to screen for it using % hypochromic cells and/or serum ferritin in early pregnancy may be a priority in antenatal care in our part of the world.

Conflict of interest:

The authors have no conflict of interests to declare.

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