Red blood cell zinc protoporphyrin measurement for assessment of peripartum iron deficiency

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Summary

Objective: To study the effectiveness of the rapid red blood cell zinc protorphyrin (RBC-ZPP) test for the detection of women with iron-deficiency anemia in the peripartum period.

Design: Blood was drawn prospectively from 150 healthy parturient women upon admission to the labor and delivery room and 72 hours after delivery. Concentration of RBC-ZPP was measured and correlated with hemoglobin level (p=0.001), mean corpuscular volume (p=0.002), hematocrit (p=0.0001), platelet count (p=0.002), and serum iron (p=0.0001), serum ferritin (p=0.0001) and serum transferrin (p=0.0001) concentrations.

Results: RBC-ZPP concentration showed a significant increase from pre-delivery to 72 hours post-delivery. This change correlated significantly with the changes in all the other parameters studied.

Conclusion: The RBC-ZPP test is a reliable, rapid, easy-to-perform, and inexpensive method of screening low-risk women, after uneventful vaginal delivery, for iron deficiency.

Introduction

The peripartum period is often characterized by a susceptibility to iron-deficiency anemia. This is attributable to the insufficient amounts of iron absorbed from food or mobilized from stores to meet the increased physiologic requirements of pregnancy and the blood loss during and after delivery, about 500-600 ml for a single fetus. This accounts for about half the erythrocytes added to the maternal circulation during pregnancy [1].

Iron supplementation, usually prescribed throughout the second half of pregnancy is beneficial in many cases, but it may be insufficient in the presence of impaired iron absorption and compliance with treatment is often poor [2]. This makes screening important during the third trimester and the early puerperium period even in normal pregnancies and uneventful deliveries.

Ferrous protoporphyrin IX, or heme, is formed by the incorporation of iron into protoporphyrin IX. When heme biosynthesis is disturbed by decreased iron availability, protoporphyrin accumulates in the RBC [3].

Since the 1930s researchers have been measuring the free erythrocyte protopophyrin (FEP) or non-heme protoporphyrin (PPN) to detect patients with depleted iron stores [3, 4]. Recent studies have demonstrated that much of the non-heme PPN in RBC of both healthy and iron deficient patients is in fact not "free" from metal cations but in the form of ZPP [5]. Extraction methods for measuring both metal-free PPN and ZPP have since been published [6].

The measurements of RBC-ZPP concentration provide information on the storage and use of iron [3]. It can be inexpensively and quickly assayed (in less than one minute) from a drop of whole blood by hematofluorometry [7]. However, published data on normal values for FEP, the performance of the hematofluorometer, and the usefulness of the ZPP test in differentiating microcytic RBC disorders in adults is scant [8, 9]. There is also little information available on the diagnostic value of this test in women with substantial blood loss during labor.

The purpose of the present study was to determine whether findings with the RBC-ZPP test are effective in the assessment of anemia risk after normal vaginal delivery and to identify women who will need continued iron therapy.

Materials and Methods

A prospective design was used to study a population of 150 low-risk parturients women attending the Labor and Delivery Unit of the Rabin Medical Center, a Tertiary Care University Hospital. All the women received standard antenatal care and completed term pregnancies, and all were prescribed oral iron supplements (30 mg/day elemental iron) beginning from week 20 of pregnancy. Patients with pre-existing iron-deficiency anemia (hemoglobin levels less than 11 g/dL), thalassemia trait, and other hemoglobinopathies were excluded, as were multiparous women (> 5 deliveries), women with a prolonged labor and delivery (more than 20 hours from admission until delivery), women undergoing operative delivery, and women with any other complication involving a clinically estimated increase in blood loss (> 500 ml) or the need for a blood transfusion. All participants provided informed consent. The study was approved by the Helsinki Committee of the Rabin Medical Center.

Venous blood samples were collected from the study participants at the time of admission to the delivery room and 72 hours after delivery. All blood measurements were carried out by the same experienced medical technician. Hemoglobin measurements were performed by the Coulter method; accuracy and precision were monitored with controls.

Red blood cell zinc protoporphyrin was measured by hematofluorometry in whole blood (Helena ProtoFluor®, Reagent

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System Kit, Cat. No. 2000). The design and evaluation of the method are given in detail elsewhere [10]. Briefly, the hematofluorometer is a dedicated front-surface fluorometer that directly measures the RBC-ZPP hemoglobin ratio as a function of the intensity of light emitted by ZPP fluorescence at 594 mm versus the amount of excitation light absorbed by hemoglobin at 420 mm. This method has a precision of 2% at the level of 80 μg/dL RBC. A value of ≥ 60 μg/dL RBC is interpreted as abnormal for women.

Statistical analysis was performed with the nonparametric Wilcoxon signed ranks test, using a commercial computer package (SPSS for Windows, Standard Version 1981-1995, SPSS Inc., Chicago, IL).

Results

Forty of the 190 women were excluded from the analysis owing to incomplete data for RBC-ZPP, serum iron, serum ferritin and serum transferrin concentrations. The remaining 150 women included 87 (58%) nulliparas and 63 (42%) multiparas, mean (± SD) age was 29.4±4.3 years. The majority were native-born Israelis (n=129, 92%), 11 (0.7%) were of Russian descent.

The values for the four red cell indices (hemoglobin level, mean corpuscular volume, hematocrit, and platelet concentrations) and for serum iron, serum ferritin and serum transferrin showed a normal distribution and a statistically significant difference between the pre-delivery and 72-hour post-delivery values (Table 1).

Concentrations of RBC-ZPP correlated significantly with all the parameters examined both before and after delivery (p<.01). Mean values, standard deviations and correlations with serum RBC-ZPP levels are shown in Table 1.

Table 1. — *Correlation between pre- and post-delivery hemoglobin and hematological characteristics and red blood cell zinc protoporphyrin (RBC-ZPP).*

	Pre-delivery	Post-delivery	p value *	Significant p value **
Hemoglobin level (g/dL)	12.0±1.0	11.1±1.4	0.000	0.000
Mean corpuscular volume (fL)	87.9±10.1	85.4±12.6	0.002	0.014
Hematocrit (%)	38.2±3.3	34.1±5.5	0.000	0.000
Platelets (x1000/μg/dL)	209.4±58.8	216.0±49.7	0.002	0.013
Serum iron (μg/dL)	64.7±15.6	58.2±36.2	0.000	0.028
Serum ferritin (ng/mL)	50.2±21.4	56.4±20.4	0.000	0.013
Serum transferrin (μg/dL)	349.1±40.8	308.2±6.1	0.000	0.000
RBC-ZPP (μg/dL)	57.7±9.6	63.1±13.2	–	0.000

Data presented as mean ± SD.
* Correlation with RBC-ZPP.
** Correlation between both pre- and post-delivery values.

Discussion

Iron-deficiency anemia may be due to deficient iron stores with inability to maintain adequate RBC mass by increasing iron absorption, or to ineffective use of normal stores. RBC-ZPP concentration is a marker for iron-deficiency anemia, with levels above 60 μg/ml signifying a decompensated state with ineffective heme synthesis [10]. It can serve as an accurate and cost-effective physiologic measure also in the peripartum period, when women are particularly susceptible, even with oral iron supplementation. Holly [8] and Romslo *et al.* [9] observed that in pregnant women who did not receive iron supplementation, RBC-ZPP concentrations rose primarily during the third trimester. This difference was not observed for ferritin levels or transferrin saturation results.

According to the findings of the present study RBC-ZPP concentrations increased significantly at 72 hours after uneventful pregnancy and term delivery from pre-delivery concentrations. This rise was compatible with the decrease in hemoglobin, hematocrit and mean corpuscular volume as well as with other hematologic indices of iron loss. Our study suggests that in the absence of preexisting anemia and hemoglobinopathies, hematofluorometer measurement of maternal RBC-ZPP at term and within 72 hours of delivery is a method for a maternal iron status. In view of its ease of use and low cost, we suggest that the RBC-ZPP assay be considered for routine use in the peripartum period in women at apparently low risk for developing iron-deficiency anemia. Such screening will help clinicians determine which patients require continued iron supplementation.

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