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BLOOD FERRITIN LEVELS IN PREGNANT WOMEN AND PREDICTION OF THE DEVELOPMENT OF FETAL INTRAUTERINE GROWTH RESTRICTION

NIVOI FERITINA U KRVI TRUDNICA I PREDIKCIJA RAZVOJA INTRAUTERINOG ZASTOJA U RASTU PLODA

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Summary: Intrauterine growth restriction is one of the leading causes of perinatal morbidity and mortality. Prediction of intrauterine growth restriction is one of the priority tasks of perinatal protection. The purpose of this study was to evaluate the levels of serum ferritin in pregnant women, which could point to a group of patients in whom possible development of fetal growth restriction could have been expected. In this investigation, we conducted a prospective study of healthy pregnant women between 30 and 32 gestational weeks, who were estimated for ferritin values. Newborn infants of low birth weight for gestational age were recorded in 8.1%. Anemia was not present in any of the patients who delivered low birth weight babies. Ferritin serum levels in mothers of the babies with low birth weight were on average 6.42 μg/L higher than in the mothers with appropriate for gestational age babies ($p<0.005$). ROC analysis of newborn infants birth weight and maternal blood ferritin levels showed that blood ferritin level had good predictive value. In case the recorded maternal blood ferritin values are above 13.6 μg/L, we can assume with the sensitivity of 64.7% and specificity of 91.7%, that the pregnant woman will develop a condition of intrauterine growth restriction. The missing decrease of ferritin values, erythrocytes, hemoglobin and hematocrit in the blood of healthy pregnant women between 30 and 32 gestational weeks, can with high probability point to the development of fetal intrauterine growth restriction.

Keywords: ferritin, pregnancy, intrauterine fetal growth restriction

Kratak sadržaj: Intrauterini zastoj u rastu ploda jedan je od vodećih uzroka perinatalnog morbiditeta i mortaliteta. Kako kauzalna terapija ne postoji, predikcija intrauterinog zastoja u rastu je jedan od prioritetnih zadataka perinatalne zaštite. Cilj rada je bio da se na osnovu određivanja nivoa serumskog feritina i elemenata crvene krvne loze kod trudnica između 30. i 32. gestacijske nedelje izdvoji grupa trudnica kod kojih je moguće očekivati nastanak zastoja u rastu ploda. Istraživanje je sprovedeno u vidu prospektivne studije među zdravim trudnicama između 30. i 32. gestacijske nedelje. Svim trudnicama određivane su vrednosti feritina. Posle porođaja utvrđen je procenat beba malih za gestacijsku dob; 8,1% rodilo je novorođenčad sa malom telesnom masom za gestacijsku dob. Anemija nije utvrđena ni kod jedne pacijentkinje koja je rodila bebu male porođajne mase a bila je prisutna kod 47,44% pacijentkinja koje su rodile bebe normalne porođajne mase. Kod majki beba male porođajne mase, nivo serumskog feritina bio je u proseku za 6,42 μg/L viši nego kod majki koje su rodile bebe normalne porođajne mase ($p<0,005$). Uporednom analizom telesne mase novorođenčadi i nivoa feritina iz krvi majke nađen je mali koeficijent korelacije, ali je ROC analiza pokazala da nivo feritina u krvi ima dobru prediktivnu vrednost. Pri vrednostima feritina u krvi trudnice većim od 13,6 μg/L sa senzitivnošću od 64,7% i specifičnošću od 91,7% možemo tvrditi da će se razviti intrauterina retardacija rasta ploda. Izostanak pada vrednosti serumskog feritina, eritrocita, hemoglobina i hematokrita u krvi zdrave trudnice u periodu između 30. i 32. nedelje trudnoće može ukazati sa velikom verovatnoćom na razvoj intrauterine retardacije rasta ploda.

Ključne reči: feritin, trudnoća, intrauterina retardacija rasta ploda

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Introduction

Ferritin is a protein, the serum concentrations of which are in correlation with total iron reserves in the human organism, and therefore it can be used as a reliable parameter in the estimation of iron deficiency

(1). Iron storage concentrations decrease with advancing gestation, hence the values of ferritin also decrease up to 32% in the first trimester, 39% in the second and even 53% during the third trimester (2). The lowest values of ferritin are recorded between 30 and 32 gestational weeks, after which the concentrations stay on constant levels. The decrease of ferritin levels is in correlation with the decrease of iron reserves in the maternal organism resulting from increased uptake (by the mother, placenta and fetus) as well as from hemodilution (3). The missing decrease of ferritin levels points to decreased extraction of iron from the blood of the pregnant woman by the fetoplacental unit, which can be in correlation with the development of intrauterine growth restriction (IUGR). Fetal intrauterine growth restriction is one of the leading causes of perinatal morbidity and mortality, following prematurity (4). The term infants of less than 2500 g of weight (below the 10th percentile) present with 5–30 times increased perinatal mortality relative to term infants of the weights corresponding to the 50th percentile (5). They are exposed to increased risks of intrapartal fetal distress, intrapartal asphyxia, neurologic developmental disorders, meconium aspiration, intrauterine death, postnatal hypoglycemia and probably the development of type 2 diabetes, obesity, autoimmune diseases, cardiovascular diseases and hypertension in adult life (6–11).

As far as there is no causal therapy, the prediction of intrauterine growth restriction is one of the priority tasks of perinatal protection.

The purpose of this study was to evaluate the levels of serum ferritin in pregnant women between 30 and 32 weeks of gestation, which could point to a group of patients in whom the possible development of fetal growth restriction could have been expected.

Material and Methods

The study was performed in the period from November 2005 to December 2006. The investigation was approved by the Ethical Comittee of the School of Medicine in Novi Sad and the Ethical Commision of the Clinical Center of Novi Sad. All the patients gave their informed consent to the study. The inclusion criteria were: 30–32 gestational-week pregnancy (estimated on the date of the last menstrual period), regular menstrual cycle, gestational week confirmed by ultrasonographic examination in the first trimester (between 8 and 13 gestational weeks), normal laboratory findings in the first and second trimester of pregnancy, term delivery. The exclusion criteria comprised: presence of chronic diseases in pregnant women (nephropathy, hypertension, ischemic cardiopathy, malignant tumors, chronic anaemia, diabetes mellitus, infection in pregnancy and smoking during pregnancy) as well as congenital malformations of the newborn. The patients provided anamnestic data and were checked for the results of laboratory analyses performed in the first and second trimester. If the values were within the limits of reference, the patients were included in the study.

Data collected from small for gestational age newborns formed the material for the study group consisting of 17 patients. A control study group was formed based on data collected from appropriate for gestational age newborns and their mothers. In the control study group were 193 patients.

All pregnant women were analyzed for blood parameters such as: erythrocytes, hemoglobin, hematocrit and leukocytes as well as the values of ferritin. The blood for blood analyses was obtained from the cubital vein on an empty stomach, before breakfast. The number of erythrocytes and leukocytes was estimated in a full blood sample with addition of an anticoagulant agent, sodium-citrate, using an automatic hematologic cell counter type Nikkon-Kohden. Hemoglobin level estimations were done from hemolysate of the obtained blood samples with the addition of sodium ferricyanide and sodium cyanide. Cyanmethemoglobin formed in the solution was estimated by spectrophotometry at the wave length of 540 nm. The level of hematocrit was also estimated by this apparatus and calculated using the following formula: Hematocrit = blood cells volume/volume of blood sample $\times$ 100.

Ferritin values were estimated by immunometric testing for quantitative determination in human serum at Olympus analyzers using the Olympus ferritin reagent (suspensions of polystyrene latex uniform size particles, lined by polyclonal rabbit anti-ferritin antibodies). Mixing of the serum containing ferritin with the Olympus ferritin reagent results in agglutination. The created immune complex in a solution disperses light depending on its size, shape and concentration, which is analyzed by spectroturbidimetry at an Olympus chemical analyzer. Reference range for our laboratory results ranges from 10.00 to 30.00 μg/L.

Vitality of the newborns was estimated by the Apgar score. Cardiac action, respirations of the newborn, muscle tonus, skin colour and reaction of the newborn are estimated by 0, 1 or 2, summed up and compared. Apgar score can vary from 0 to 10.

Statistical data analysis was performed using a Statsoft Statistica programme package.

Results

Out of 210 pregnant women who completed the investigation, 17 (8.1%) gave birth to infants of small for gestational age birth weight (birth weight less than 10th percentile adjusted for gestational age), whereas 193 (91.9%) delivered infants appropriate for gestational age.

Table I Mean (SD) of the variables studied in pregnant women with and without low birth weight.

	Control group	Study group	Significance
Mother's age (years)	28.0 (5.67) 95% CI 27.2–28.9	27.8 (5.7) 95% CI 22.1–28.2	p>0.05
Gestational age (days)	271 (7.68) 95% CI 270–272	267 (6.13) 95% CI 264–270	p>0.05
Erythrocytes ($x10^{12}$/L)	3.45 (0.29) 95% CI 3.41–3.49	3.93 (0.23) 95% CI 3.82–4.02	p<0.005
Hemoglobin (g/L)	112.38 (13.23) 95% CI 110.52–114.24	128 (9.69) 95% CI 123.4–132.6	p<0.005
Hematocrit	32.91 (2.59) 95% CI 32.55–33.27	35.65 (2.83) 95% CI 34.3–36.98	p<0.001
Leukocytes ($x10^9$/L)	10.95 (1.91) 95% CI 10.69–11.21	12.58 (1.92) 95% CI 11.67–13.49	p<0.005
Ferritin (μg/L)	11.11 (3.24) 95% CI 10.65–11.57	17.54 (7–84) 95% CI 13.81–21.25	p<0.005
Birth weight (g)	3437.5 (448.5) 95% CI 3373.8–3501.1	2304.11 (177) 95% CI2213.1–2395.1	p<0.001
Body length (cm)	50.2 (1.8) 95% CI 49.9–50.4	45.5 (1.2) 95% CI 44.8–46.1	p<0.001
Apgar score 1st min	9.3 (0.79) 95% CI 9.19–9.41	9.2 (0.53) 8.95	p>0.05
Apgar score 5th min	9.7 (0.49) 95% CI 9.63–9.77	9.6 (0.49) 95% CI9.37–9.83	p>0.05

Anemia (Hgb<110 g/L) was not recorded in the patients who gave birth to small birth weight infants, whereas it was present in 47.44% patients with appropriate for gestational age babies.

There were no stastistical differences in the pregnant women's age and gestational age of pregnancy between the two investigated groups (p>0.05). There were also no statistical differences in the Apgar score in the 1st and 5th minute (*Table I*).

In the small for gestational age group, there were 11 female (64.71%) and 6 male (35.29%) newborns. The overall sample comprised 107 female and 103 male newborn infants. The difference obtained by the anlaysis of the χ^2 test was not statistically significant.

The values of erythrocytes and hemoglobin were statistically significantly increased in the patients with small for gestational age birth weight babies (p<0.005) (*Table I*). The mean leukocyte levels (12.6 × 10^9/L) in the small for gestational age group infants were statistically significantly increased compared with the control group (10.95 × 10^9/L) (p<0.05); presence of infection was previously excluded in both groups by estimating CRP value as an early marker of infection. Serum ferritin levels in mothers with low birth weight babies were on average 6.42 μg/L higher than in the mothers who delivered appropriate for gestational age babies (p<0.005) (*Table I*). The mean birth weight in small for gestational age newborns was 2304.11 g whereas the mean birth weight of the babies in the control group was 3437.5 g (p<0.001). Differences in the Apgar score values at 1 min (9.3 vs 9.2) and 5 min (9.7 vs 9.6) between the newborn infants of the control group and the group of small for gestational age newborns, were not statistically significant (p>0.05) (*Table I*).

There was a correlation between the newborn birth weight and maternal blood ferritin levels, but of a low degree (correlation degree r = –0.24) (P=0.003) (*Figure 1*).

On the basis of our sample, we also analyzed the sensitivity and specificity of different serum feritin values and we established a decision threshold of 13.6 μg/L (*Table II*). ROC prediction analysis of the decreased birth weight term newborn infants through the use of maternal serum ferritin (30–32 gestational weeks) showed a value of 0.78 (95% CI 0.72–0.83) pointing to very good predictive capability of the ferritin level values (p<0.0001) (*Figure 2*).

By use of the logistic regression method i.e. including the effect of several variables (the values of ferritin, erythrocytes, hemoglobin) on giving birth to decreased birth weight term infants, for the cases of high statistical significance (p<0.05), we obtained significance levels for each controlled variable (*Table III*).

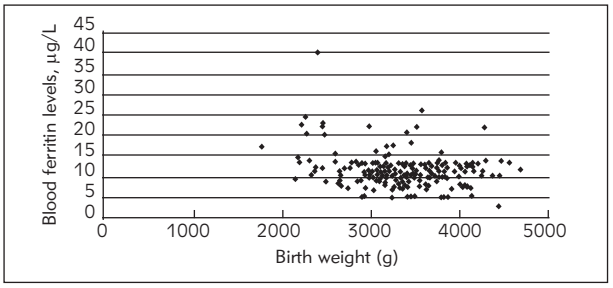

Figure 1 Correlation of birth weight (g) and blood ferritin levels (μg/L) in pregnant women between 30 and 32 weeks of gestation.

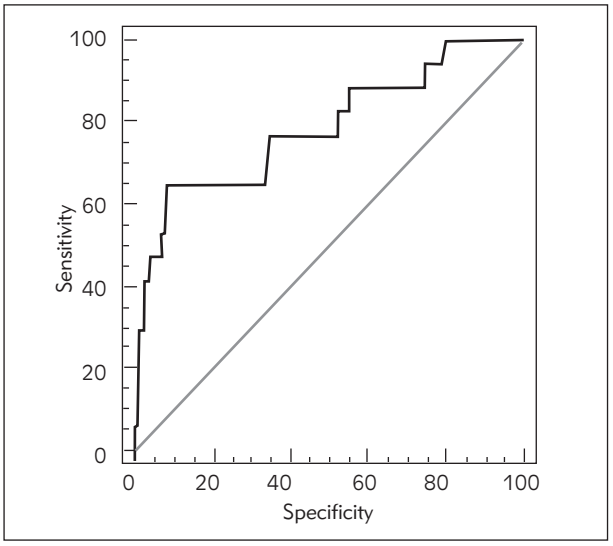

Figure 2 ROC curve with the specificity/sensitivity ratio of blood ferritin levels in pregnant women.
ROC prediction analysis of the decreased birth weight term newborn infants through the use of maternal serum ferritin (30–32 gestational weeks) showed the value of 0.78, pointing to a very good predictive capability of ferritin level values ($p<0.0001$).

Table II Sensitivity and specificity of blood ferritin levels.

Blood ferritin levels (μg/L)	Sensitivity (%)	Specificity (%)
>5	100	1
>7	100	8.5
>9	94.1	21.2
>11	82.4	47.2
>13.6 *	64.7	91.7
>15	47.1	93.8
>17	43.1	96.2

* level of significance

Table III Value of variables according to the logistic regression method.

Variable	Coefficient	SD	p
Ferritin	0.160	0.067	0.0154
Erythrocytes	3.610	1.110	0.0012
Hemoglobin	0.073	0.025	0.0039

SD – standard deviation; p – level of significance

Discussion

Of the 220 pregnant women with a gestational age of 30–32 weeks included in the investigation, 10 were excluded during the investigation period for the following reasons: 3 pregnant women developed hypertension at 32 gestational weeks, 2 presented with gestational diabetes, 4 women delivered before the 37th completed week of gestation, whereas 1 blood sample was contaminated by the presence of fibrin and estimations of ferritin could not have been done. Out of 210 pregnant women who completed the investigation, 17 (8.1%) delivered newborn infants of the birth weight small for gestational age, which was in agreement with the results of other authors in which the incidence of newborn infants with small for gestational age birth weight in investigated sample was 3.3–10% in the developed i.e. 6.7–17% in developing countries (1, 12, 13).

The values of erythrocytes and hemoglobin were statistically significantly increased in the patients with small for gestational age birth weight babies ($p<0.005$). The increased value of hematocrit was statistically highly significant in the patients with small for gestational age birth weight babies ($p<0.001$). The values of erythrocytes and hemoglobin and hematocrit in maternal blood of the control group and the small for gestational age group were similar to the results reported by other authors (14–17).

Madhavan Nair et al. (14) found a significant increase in serum ferritin and a decrease in hemoglobin in non-anemic pregnant women. Langini et al. (15) found the placental ferritin concentration lower in women with hemoglobin <110 g/L than in women with normal values: 26±13 vs. 38±20 μg/g, respectively ($p=0.021$).

The mean values of leukocytes were 12.6×10^9/L in the small for gestational age birth weight vs. 10.95×10^9/L in the control group. The purpose of leukocyte estimations was to exclude the presence of infection of any etiology. Infection provokes an increase in the levels of ferritin due to increased excretion of inflammatory cytokines inducing the synthesis of ferritin, and possibly giving false-positive results. For that reason infection was previously excluded in both groups by estimating CRP value as an early marker of infection. Some similar studies did not comprise estimations of the levels of leukocytes (1).

According to the up to now reported studies, pregnant women with small for gestational age birth weight infants present with increased levels of hemoglobin, hematocrit, erythrocytes and ferritin compared with the pregnant women with appropriate for gestational age infants. The recorded ferritin values in pregnant women with IUGR were above 15 μg/L, whereas blood ferritin values in pregnant women with appropriate for gestational age infants were below 15 μg/L (1, 18). The mean value of ferritin in our sample was 17.53 μg/L in the group of pregnant women with small for gestational age birth weight infants, whereas it was 11.11 μg/L in the control group.

Ferritin concentrations in maternal blood vary, depending on the level of extraction by the feto-placental unit. Pregnant women with IUGR infants have increased blood ferritin levels because of the decreased extraction of iron and ferritin by the placenta (1). Some authors believe that fetal IUGR leads to a reduction of placental perfusion, separation of small parts of the placenta and some other aspects of placental pathology, so that damage to placental parenchyma, which is significantly rich in ferritin, leads to an increase in maternal serum ferritin concentrations on the one hand, and on the other to a decreased extraction of ferritin both from the placenta and the fetus (19, 20). As far as the decrease in iron extraction precedes clinical development of IUGR, it is believed that the control of ferritin levels in maternal blood can be a reliable parameter in the prediction of fetal intrauterine growth restriction, if the expected decrease in ferritin levels does not occur during the third trimester of pregnancy. It is considered that the women with ferritin concentrations above 15 μg/L at 30–32 gestational weeks have 4.5 times increased risk of giving birth to small birth weight term infants (1). Iron deficiency and fetal anemia lead to increased synthesis of corticotropic releasing hormone, causing increased secretion of fetal cortisol, inducing inhibition of fetal growth (21).

Similar results for the values of erythrocytes, hemoglobin and ferritin were found in other studies (22–26).

Our investigation showed the presence of a weak correlation between the values of newborn birth weight and maternal ferritin levels estimated between 30 and 32 gestational weeks, which was expected when considering the literature reports.

According to our sample, the optimal decision threshold for pregnant women blood ferritin values in the period from 30 to 32 gestational weeks is >13.6 μg/L. These values confirm, with 64.7% sensitivity and 91.7% specificity, the development of intrauterine growth restriction. Similar results were presented by other authors' investigations (1, 23–25). The value of 0.78 (95% CI 0.72–0.83) achieved by the ROC analysis of prediction of low birth weight in term newborn infants using the ferritin levels obtained from maternal blood in the period between 30 and 32 gestational weeks, points to the very good predictive value of ferritin level estimations ($p<0.0001$).

According to the logic regression method, i.e. the effect of multiple variables (the values of ferritin, erythrocytes, hemoglobin) on the development of term newborn infants small for gestational age relative to the cases of high statistical significance ($p<0.05$), the following levels of significance were obtained: ferritin value $p=0.0154$, erythrocyte count $p=0.0012$ and hemoglobin $p=0.0039$ (*Table II*).

The results obtained in the investigation suggest that estimations of serum ferritin values and of the red blood count elements in pregnant women represent a useful and good indicator of placental trophoblastic activity. The missing decrease of ferritin values in the blood of healthy pregnant women recorded in the period between 30 and 32 gestational weeks i.e. at serum ferritin levels of > 13.6 μg/L, can point to impaired transfer of micronutrients from the mother to fetus, which with high probability leads to the development of fetal intrauterine growth restriction.

Conflict of interest statement

The authors stated that there are no conflicts of interest regarding the publication of this article.

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