

Prohepcidin and its possible role in anaemia of pregnancy

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Abstract

Introduction: Hepcidin is the predominant negative regulator of iron absorption in the small intestine, iron transport across the placenta, and iron release from macrophages. The aim of the study was to assess prohepcidin levels in pregnant women in relation to anaemia, inflammatory cytokines and iron status.

Material and methods: The studies were performed on 37 healthy pregnant women (third trimester), 34 anaemic pregnant women (third trimester), 20 healthy pregnant women (first trimester) and 30 healthy female volunteers. Prohepcidin, hsCRP, soluble receptor of transferrin (sTfR) and interleukin-6 were studied using commercially available kits.

Results: Prohepcidin was higher in pregnancy, namely in anaemic patients, than in healthy females. In anaemic pregnant females prohepcidin showed a correlation with haemoglobin, haematocrit and total protein. In pregnant females in the first trimester prohepcidin was related to hsCRP. In all non-anaemic pregnant women (first and third trimester) prohepcidin correlated with sTfR and ferritin. In healthy volunteers prohepcidin was related to ferritin.

Conclusions: Elevated prohepcidin in pregnancy related to ferritin and sTfR may reflect an acute phase reaction, and either the cellular need for iron or the rate of erythropoiesis. In anaemia in pregnancy a cause-effect relationship between haematocrit and hepcidin might exist. A rise in prohepcidin in pregnancy may also be due to the fact that some proinflammatory cytokines play a fundamental role in inducing hepcidin gene expression, namely IL-6.

Key words: prohepcidin, inflammation, pregnancy, anaemia, cytokines.

Introduction

Normal pregnancy is characterized by profound changes in almost every organ system to accommodate the demands of the fetoplacental unit. A greater increase in intravascular volume compared to erythrocyte mass results in the dilutional/physiological anaemia of pregnancy, most apparent at 30 to 34 weeks of gestation. Maintaining the correct iron balance is crucial for health. Our understanding of the molecular control of iron metabolism has increased dramatically over the past 5 years due to the discovery of hepcidin. Overexpression of the hepcidin gene resulted in mice that were severely iron deficient at birth [1]. Hepcidin is the predominant negative regulator of iron absorption in the small intestine, iron transport across the placenta, and iron release from macrophages [2]. High levels of hepcidin/prohepcidin have been found in patients with anaemia of chronic disease [3]. Based on these findings the aim of the study was to assess

prohepcidin levels in pregnant women in relation to anaemia, inflammatory cytokines and iron status.

Material and methods

The studies were performed on 37 healthy pregnant women (third trimester), 34 anaemic pregnant women (third trimester), 20 healthy pregnant women (first trimester) and 30 healthy female volunteers. All patients were informed about the aim of the study and gave their consent. The study was approved by the Ethics Committee at the Doctors' Council (Izba Lekarska) in Olsztyn. All the pregnancies were uneventful. There were no miscarriages in the pregnant females (studied at first trimester). In none of the pregnant females in advanced pregnancy was hypertension, gestational diabetes, hypotrophy or preterm labour observed. Laboratory tests were done at week 9 in pregnant females (first trimester) and at week 36 in pregnant females (third trimester). All the females were given folic acid. Pregnant females were also given iron supplements starting from confirmation of pregnancy (in the form of multivitamin supplements). Anaemia was diagnosed at the beginning of the third trimester and iron supplements in the form of oral iron were introduced.

Since prohepcidin is affected by kidney function we included only females with normal kidney function [4]. Blood was taken without stasis. Prohepcidin was studied using commercially available kits from DRG Instruments GmbH (Marburg, Germany) and CRP was assayed by a high sensitivity method using commercially available kits from American Diagnostica (Greenwich, CT, USA). Soluble receptors of transferrin (sTfR) and interleukin-6 (IL-6) were studied using kits from R&D (Abington, UK). All tests were performed according to manufacturers' instructions by the same person. The following parameters were measured by means of standard laboratory methods: haemoglobin, red blood cell count, total protein, albumin, cholesterol, triglycerides, and urea. Transferrin saturation (TSAT) was calculated as iron/total iron binding capacity (TIBC) ratio. Serum iron and other parameters (TIBC, TSAT, ferritin) were assessed after a one-week break in oral iron supplementation to avoid interpretation bias.

Data were analyzed using Statistica 6.0 computer software. Normality of variable distribution was tested using Shapiro-Wilk W-test. If possible data were logarithmically transformed to achieve normal distribution. Data were reported as means \pm SD. Analysis of variance (ANOVA) (with post-hoc Tukey test for unequal groups) or Kruskal-Wallis ANOVA (the difference between the means of two variables was calculated by Mann-Whitney U test) was used to compare differences between groups with $P < 0.05$ considered statistically significant, when appropriate. Linear regression analysis employed Pearson or Spearman

coefficients as appropriate. Multiple regression analysis was used to determine independent factors affecting dependent variables. Factors showing linear correlations with prohepcidin ($P < 0.01$) were included in the multiple regression analysis.

Results

All the data are presented in Table I-III. In pregnant anaemic women in the third trimester prohepcidin, IL-6, hsCRP, sTfR, TIBC and TSAT were significantly higher than in non-anaemic pregnant women in the same trimester. Prohepcidin in every trimester was significantly higher than in non-pregnant healthy females. In anaemic pregnant females prohepcidin showed negative correlations with haemoglobin ($r = -0.34$, $P < 0.05$), haematocrit ($r = -0.39$, $P < 0.05$), and total protein ($r = -0.34$, $P < 0.05$). In pregnant females in the first trimester prohepcidin was positively related to hsCRP ($r = 0.49$, $P < 0.05$). In all non-anaemic pregnant women (first and third trimester) prohepcidin correlated negatively with sTfR ($r = -0.27$, $P < 0.05$) and positively with ferritin ($r = 0.26$, $P < 0.05$). In healthy volunteers prohepcidin was related positively to ferritin ($r = 0.37$, $P < 0.05$).

Discussion

In uncomplicated iron deficiency anaemia, both the anaemia per se and the absent iron stores provide a message to stop production of hepcidin, a protein produced in the liver which is felt to be a major regulator of iron balance. The absence of hepcidin enhances gastrointestinal iron absorption as well as the release of iron from stores in macrophages. Further loss of iron results in anaemia, which is initially normocytic with a normal absolute reticulocyte count. This stage of iron deficiency is common. It has been estimated that more than 20% of menstruant women in the United States have no iron reserves and are in this stage [5].

In our studies we found that prohepcidin was significantly higher in pregnant women than in healthy females, in contrast to the recent study of Roe et al. [6]. We studied a much larger population of pregnant females: 91 pregnant females, not only 13, as reported previously. In addition, we chose a control group of the same age, sex and kidney function to avoid potential bias. Roe et al. [6] compared hepcidin levels to those obtained in men over 40 years. The values obtained in our control group correspond to those reported by Kulaksiz et al. [7]. Moreover, in our study, prohepcidin was significantly higher in anaemic pregnant females than in non-anaemic in the third trimester. In the study of Laskowska-Klita et al. [8] prohepcidin was not affected by the duration of the pregnancy; however, they obtained values much higher than Roe et al. [6]. They did not provide reference values. Roe et al. [6] found that in 6 pregnant females iron supplement-

Table I. Basal clinical and biochemical characteristics of the studied groups

	3 rd trimester anaemic (n=34)	3 rd trimester non-anaemic (n=37)	1 st trimester non-anaemic (n=20)	Healthy volunteers (n=30)
Age [years]	26.85±5.05 (17-37)	26.35±5.91 (18-41)	26.90±5.12 (19-39)	30.01±8.33 (21-41)
Number of pregnancies	2.17±1.47 (1-7)	3.19±1.63 (1-4)	1.63±0.76 (1-3)	2.02±0.95 (1-3)
Ht [%]	29.95±2.15 ^{***##} (24.0-32.0)	35.99±2.19 ^{***} (35.8-42.2)	38.59±2.86* (33.0-40.2)	39.87±2.48 (37.0-46.8)
Hb [g/dl]	9.90±0.92 ^{***##} (6.9-10.9)	12.13±0.78 ^{**} (11.0-14.0)	12.98±0.99 (11.3-13.9)	13.87±0.94 (12.1-16.7)
Erythrocyte count [$\times 10^9/\mu\text{l}$]	3.49±0.33 ^{***##} (2.55-4.08)	3.88±0.27 ^{**} (3.39-4.44)	4.18±0.29 (3.64-4.62)	4.24±0.29 (4.06-5.15)
Leukocyte count [$\times 10^3/\mu\text{l}$]	9.37±2.42 ^{***} (6.0-16.1)	10.28±2.12 ^{***} (6.4-16.5)	8.22±1.56 (4.8-12.0)	5.53±1.61 (4.9-9.8)
MCV [fl]	86.34±7.48 (69.9-105.5)	92.68±4.67 (83.2-102.9)	87.76±3.46 (82.2-98.9)	85.76±3.87 (82.4-92.5)
Platelet count [$\times 10^3/\mu\text{l}$]	214.18±54.05* (125-322)	206.62±41.91* (135-279)	238.21±63.48 (141-390)	241.45±44.55 (140-295)
Total protein [g/dl]	5.82±0.61 (4.9-7.3)	5.94±1.41 (4.9-6.9)	5.84±1.41 (5.5-7.2)	ND
Creatinine [mg/dl]	0.58±0.12 (0.43-0.9)	0.63±0.13 (0.44-0.87)	0.67±0.09 (0.55-0.87)	0.77±0.14 (0.6-0.99)
INR	0.99±0.08 (0.86-1.14)	0.97±0.10 (0.6-1.2)	1.11±0.08 (0.93-1.29)	ND
APTT	34.60±5.61 (23.2-43.4)	35.27±11.45 (25.7-87.9)	34.74±6.38 (25.2-50.9)	ND
PT	101.47±6.49 (89.2-112.2)	102.45±5.82 (88.6-114.7)	91.49±4.54 (80.0-98.8)	ND

Values given are means \pm SD, ND – not done

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. control group

$P < 0.05$, ## $P < 0.01$ 3rd trimester anaemic vs. 3rd trimester non-anaemic

Table II. Parameters of iron metabolism and inflammation in the studied groups

	3 rd trimester anaemic (n=34)	3 rd trimester non-anaemic (n=37)	1 st trimester non-anaemic (n=20)	Healthy volunteers (n=30)
Iron [$\mu\text{g/dl}$]	84.35±52.84* (23-230)	89.75±27.47 (41-150)	101.58±31.28 (38-196)	111.34±24.65 (52-195)
TIBC [$\mu\text{g/dl}$]	537.62±92.99 ^{##} (399-702)	494.18±77.68 (366-666)	341.87±51.6 (258-471)	321.76±88.76 (228-460)
TSAT [%]	15.69±5.68 ^{***} (6-33)	18.16±3.15 ^{***} (11-23)	29.71±6.06* (15-42)	34.60±2.77 (23-40)
Ferritin [ng/ml]	26.94±29.35 (3.6-186.8)	30.23±32.45 (4.5-99.5)	30.55±19.41 (7.22-70.16)	26.78±15.42 (9.8-81.8)
Prohepcidin [ng/ml]	234±30.54 ^{***} (90-490)	193±20.65 ^{**} (81-313)	184±22.87 ^{**} (41-286)	98.76±18.76 (28-163)
sTfR [nmol/l]	29.42±15.80 ^{##} (12.2-67.7)	21.74±7.90 (11.0-46.8)	17.81±6.81 (11.2-32.5)	16.65±3.31 (9.6-26.2)
hsIL 6 [pg/ml]	9.10±3.49 ^{***} (4.3-18.9)	5.79±3.18* (2.62-16.0)	5.18±2.75 ^{**} (1.0-10.4)	2.26±2.02 (0.3-6.8)
hsCRP [mg/ml]	7.54±2.89 ^{***} (2.54-13.36)	5.49±3.71 ^{**} (0.05-11.8)	2.71±1.88 (0.05-6.3)	1.74±0.52 (0.05-2.49)

Values given are means \pm SD, ND – not done

* $P < 0.05$, ** $P < 0.001$, *** $P < 0.001$ vs. control group

$P < 0.05$, ## $P < 0.01$ 3rd trimester anaemic vs. 3rd trimester non-anaemic

tation did not affect prohepcidin levels. All of our patients were on multivitamin supplements including iron from the diagnosis of pregnancy, not from the 16th week.

Measurement of serum ferritin is currently the accepted laboratory test for diagnosing iron deficiency, and a ferritin value of 12 $\mu\text{g/l}$ is a highly specific indicator of iron deficiency [9]. Serum ferritin is useful in pregnant women who often have elevated serum transferrin in the absence of iron deficiency [10]. Other commonly used laboratory tests such as serum iron, total iron-binding capacity, mean corpuscular volume, and transferrin satu-

ration provide little additional diagnostic value over ferritin. Measurement of serum iron and percent saturation of TIBC had significantly lower diagnostic accuracy. The accuracy of measurement of transferrin/TIBC for predicting the presence of iron deficiency is second only to the serum or plasma ferritin concentration. One problem is that pregnancy and oral contraceptives increase the plasma transferrin concentration [10]; as a result, the percent saturation may be low in such patients in the absence of iron deficiency.

The soluble transferrin receptor, a truncated form of the membrane-associated transferrin receptor,

Table III. Comparison between pregnant females with and without anaemia

	3 rd trimester anaemic (n=34)	3 rd trimester non-anaemic (n=37)	P
Age [years]	26.85±5.05	26.35±5.91	NS
Number of pregnancies	2.17±1.47	3.19±1.63	NS
Ht [%]	29.95±2.15	35.99±2.19	<0.01
Hb [g/dl]	9.90±0.92	12.13±0.78	<0.01
Erythrocyte count [$\times 10^6/\mu\text{l}$]	3.49±0.33	3.88±0.27	<0.05
Iron [$\mu\text{g/dl}$]	84.35±52.84	89.75±27.47	NS
TIBC [$\mu\text{g/dl}$]	537.62±92.99	494.18±77.68	<0.05
Ferritin [ng/ml]	26.94±29.35	30.23±32.45	NS
Prohepcidin [ng/ml]	234±30.54	193±20.65	<0.05
sTfR [nmol/l]	29.42±15.80	21.74±7.90	<0.05
hsIL 6 [pg/ml]	9.10±3.49	5.79±3.18	<0.05
hsCRP [mg/ml]	7.54±2.89	5.49±3.71	NS

NS – non statistical

has been reported to be a sensitive indicator of iron deficiency and is not an acute-phase reactant [11]. Therefore, in our study we also assessed sTfR and its possible correlation with prohepcidin. In our study sTfR was related to prohepcidin in all non-anaemic pregnant females.

In anaemic pregnant females prohepcidin correlated with haematocrit. In the recent study of Hsu et al. [12] the only predictor of prohepcidin in haemodialyzed patients was haematocrit, whereas in univariate analysis significant correlations between prohepcidin and IL-6, serum erythropoietin level and dose were observed. They did not discriminate between anaemic and non-anaemic patients. In all non-anaemic pregnant women prohepcidin was related to ferritin, as reported by Dallalio et al. [13] for anaemic patients undergoing bone marrow examinations. Kulaksiz et al. [7] reported elevated prohepcidin level in haemodialyzed patients. All of their 59 patients were treated 2-3 times a week with 3000 IU erythropoietin. However, they did not provide even basal clinical characteristics of studied patients, not even haemoglobin level. They only stated that patients with renal anaemia (a maximum haemoglobin concentration of 11 g/dl) had significantly lower hepcidin than patients with chronic renal insufficiency. No correlations between hepcidin, iron status, ferritin and transferrin saturation were observed in their study. In our previous study we found significantly higher hepcidin in non-anaemic HD patients treated with erythropoietin when compared to anaemic HD patients treated with erythropoietin [14]. In patients treated with erythropoietin in multiple regression analysis the only correlates of hepcidin were haemoglobin and erythropoietin dose. In our study pregnant

females were given iron supplements, despite in some of them anaemia being diagnosed in the third trimester. It seems that anaemia in pregnancy is not only due to iron deficiency but also to subclinical inflammation as reflected by elevated IL-6 and CRP. According to the Centers for Disease Control and Prevention (CDC) pregnant women should begin taking low dose (30 mg/day) oral iron at the first prenatal visit as primary prevention of iron deficiency. Women should also be screened for iron deficiency at the first prenatal visit; the presence of anaemia warrants treatment with 60 to 120 mg of iron per day. Iron is best absorbed from an empty stomach in the reduced or ferrous form [15].

In conclusion, hepcidin is essentially a microbicidal peptide. Assuming that in certain conditions the body is particularly vulnerable (pregnancy, chronic kidney disease, inflammation), increased hepcidin synthesis in the liver will protect the organism against bacterial or microbial aggression or dissemination. However, the price to pay could be anaemia. Elevated hepcidin in pregnancy related to ferritin and sTfR may reflect an acute phase reaction, and either the cellular need for iron or the rate of erythropoiesis. In anaemia in pregnancy a cause-effect relationship between haematocrit and hepcidin might exist. A rise in prohepcidin in pregnancy may also be due to the fact that some proinflammatory cytokines play a fundamental role in inducing hepcidin gene expression, namely IL-6 [3].

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