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Serum Prohepcidin Concentrations In Preeclamptic Pregnant Women: An Analysis Concerning Serum Iron Status Markers and Compared To Healthy Pregnant Women

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ABSTRACT

This study aimed to evaluate serum prohepcidin concentrations in preeclamptic pregnant women related to iron status markers and compared to healthy pregnant women. A total of 80 pregnant women admitted to a tertiary care obstetric clinic were included in this cross-sectional study. Pregnant women were divided into two groups based on preeclampsia screening results, including those with preeclampsia (n=40) and healthy pregnant women (n=40). Data on the gestational week at the time of blood sampling, and blood analysis findings including complete blood count (CBC), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), iron homeostasis parameters (serum iron, ferritin, and total iron-binding capacity [TIBC]), and plasma prohepcidin levels were recorded in each woman. The presence of preeclampsia was associated with significantly higher WBC count (13.5±4.4 vs. 10.9±2.9 x103/µL, p=0.001) and serum iron (102.7±65.0 vs. 74.8±54.0 µmol/L, p=0.04) and ferritin (40.9±41.2 vs. 18.6±29.1 µg/L, p=0.007) and plasma prohepcidin (38.2±39.3 vs. 17.91±14.71, p=0.004) levels compared to the healthy pregnant woman. Our findings indicate an increase in serum iron and ferritin levels coupled with elevated prohepcidin levels in pregnancies complicated by preeclampsia. Further longitudinal researches are needed to clarify the role of prohepcidin and active hepcidin in preeclampsia and the potential mechanisms underlying the unregulated hepcidin-iron homeostasis.

Keywords: Preeclampsia; prohepcidin; serum iron status markers

Introduction

Preeclampsia complicates about 5-8% of pregnancies and is characterized by hypertension, proteinuria, and vascular abnormalities that develop in the third trimester and leads to maternal and fetal morbidity and mortality (1-4). Vasoconstriction, placental ischemia-reperfusion injury mediated reactive oxygen species (ROS) generation, oxidative stress, and endothelial injury play a significant role in preeclampsia pathogenesis (1,4-6).

Given the increase in transferrin saturation, serum iron, and ferritin levels in pregnancies complicated by preeclampsia, alterations in iron homeostasis has been proposed to contribute to the pathogenesis in preeclampsia by the iron-overload induced generation of ROS that exacerbates oxidative stress and endothelial injury and thus can worsen the ongoing pathological complications of preeclampsia (5,7,8).

Hepcidin is a recently described acute phase peptide produced by the liver in response to iron overload and inflammatory stimuli and acts as a homeostatic regulator of systemic iron metabolism and a link between iron homeostasis and host defense (8-10). Hepcidin reduces the cellular free iron and iron-mediated cytotoxicity and oxidative stress via

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downregulating ferroportin and upregulating ferritin expression in cells (6).

Iron accessibility is crucial during gestation for both the mother and the growing fetus. Hepcidin regulates systemic iron bioavailability. Alterations in prohepcidin and hepcidin levels are considered likely in preeclampsia. In contrast, hepcidin's role in iron homeostasis in preeclamptic pregnant women remains unclear with the limited number of studies to date addressed hepcidin levels concerning iron homeostasis in preeclamptic pregnancies (6,8-10).

Therefore, we designed this study to evaluate prohepcidin concentrations in preeclamptic pregnant women related to iron status markers and compared to healthy pregnant women.

Materials and Methods

Subject population: A total of 80 pregnant women admitted to a tertiary care obstetric clinic were included in this cross-sectional study. All the pregnant women were in the age range of 18-40 years and 20-34 weeks of gestation. Pregnant women were divided into two groups based on the American College of Obstetricians and Gynecologists (ACOG) preeclampsia screening (11), including those with preeclampsia (n=40) as the study group and normotensive healthy gestational age-matched and body mass index (BMI)-matched patients without proteinuria as the control group (n=40). The control group consisted of pregnant women who did not experience any complications associated with pregnancy in the later stages of pregnancy and had given birth at term.

We excluded patients with eclampsia, HELLP syndrome, ruptured amniotic membranes, multiple pregnancies, hepatic disease, fetal chromosomal abnormalities, congenital fetal malformations, stillbirths, anemia, infections, fever, patients at the active phase of labor, patients with a history of gestational hypertensive disorders, and co-existing morbidities, including chronic hypertension, collagen vascular disease, renal disease, diabetes mellitus, hypothyroidism, ischemic heart disease, and known malignancy.

Written informed consent was obtained from all participants. The institutional ethics committee approved the study (Date of Approval: 10/10/2013, Reference number/Protocol No: 66519339/16143).

Assessments: We collected blood and urine samples of the patients at the PE diagnosis period for the study group and routine antenatal care before labor for the control group. Data on BMI (kg/m^2), systolic blood pressure (BP) (mmHg), diastolic BP (mmHg), gestational week at the time of blood sampling, and blood analysis findings including complete blood count (CBC), erythrocyte sedimentation rate (ESR, mm/h), C-reactive protein (CRP, mg/L), iron homeostasis parameters (serum iron, ferritin, and total iron-binding capacity [TIBC, μmol/L]) and plasma prohepcidin (ng/mL) levels were recorded in each woman.

Blood biochemistry: CBC (Abbott Cell-Dyn 3700SL, Abbott Laboratories, Diagnostic Division, Illinois, USA), ESR, CRP (ELISA method, BNII, Dade Behring, Marburg GmbH, USA), iron and TIBC (Aeroset System Abbott, Abbott Laboratories, Diagnostic Division, Illinois, USA) and ferritin (DPC Immulite 2000, Scientific Affairs, DPC Biermann, Germany) were determined according to routine laboratory analyses.

For the measurement of prohepcidin, a serum aliquot was frozen immediately at -40°C, and serum levels of prohepcidin (ng/mL) were determined in batch using a commercial ELISA kit (BNII, Dade Behring, Marburg GmbH, USA) according to the manufacturer's protocol.

Statistical Analysis: Statistical analysis was made using IBM SPSS Statistics for Windows, version 20.0 (IBM Corp., Armonk, NY). We presented the measured variables as mean±standard deviation (std), and categorical variables as numbers and percentages (%). The Kolmogorov-Smirnov test was used to determine whether the numerical data matched the normality distribution. Analysis of numerical variables was performed via Student's t-test or Mann-Whitney U test. We considered the p-value <0.05 as statistically significant.

Results

We presented the demographic and clinical characteristics in Table 1. There were no significant differences between the two groups in terms of maternal age, gravidity, parity, BMI, and gestational age at blood sample collection.

We summarized the CBC and blood biochemistry test results of the patients in Table 2. The presence of preeclampsia was associated with significantly higher WBC count (13.5±4.4 vs. 10.9±2.9 x103/μL, p=0.001), serum iron (102.7±65.0 vs. 74.8±54.0 μmol/L, p=0.04), serum ferritin (40.9±41.2 vs. 18.6±29.1 μg/L, p=0.007) and serum prohepcidin (38.2±39.3 vs. 17.91±14.71, p=0.004) levels, whereas with significantly lower ESR (42.2±22.0 vs. 54.5±15.7, p=0.005) compared to the healthy pregnant women.

Table 1. The Demographic and Clinical Characteristics of The Patients

	Preeclampsia group (n=40)	Control group (n=40)	P-value
Maternal age, years	27.7 ± 6.3	26.4 ± 5.2	0.86
Gravidity	2.64 ± 1.47	2.84 ± 1.71	0.79
Parity	1.48 ± 1.23	1.66 ± 1.39	0.58
Body Mass Index, kg/m2	24.8 ± 3.3	24.2 ± 4.1	0.94
Gestational age at blood sample collection, weeks	31.2 ± 5.6	32.1 ± 2.6	0.33
Systolic Blood Pressure, mmHg	161.5 ± 16.3	111.8 ± 12.2	**<0.001**
Diastolic Blood Pressure, mmHg	98.8 ± 6.9	68.0 ± 9.7	**<0.001**
Birth week	32.6 ± 4.3	38.43 ± 1.2	**<0.001**
Proteinuria, mg/24-hour	4865.14 ± 3341.83	N/A	N/A

Measured variables were presented as mean±standard deviation. For statistical analysis, Kolmogorov-Smirnov test, Student's t-test, and Mann-Whitney U test were used

Table 2. Cbc and Blood Biochemistry Findings of The Participants

	Preeclampsia group (n=40)	Control group (n=40)	P-value
WBC (x103/μL)	13.5 ± 4.4	10.9 ± 2.9	**0.001**
Thrombocyte (x103/μL)	226.7 ± 84.8	226.3 ± 63.3	0.97
Hemoglobin (g/L)	13.4 ± 10.7	11.3 ± 1.3	0.21
Hematocrit (%)	36.00 ± 4.77	36.1 ± 3.2	0.89
MCV (fL)	86.2 ± 8.9	88.3 ± 7.6	0.24
MCH (pg)	27.9 ± 3.6	27.5 ± 3.0	0.67
MCHC (g/dL)	32.2 ± 1.4	31.4 ± 1.5	**0.012**
Serum iron (μmol/L)	102.7 ± 65.0	74.8 ± 54.0	**0.04**
TIBC (μmol/L)	375.9 ± 100.1	398.0 ± 68.8	0.25
Ferritin (μg/L)	40.9 ± 41.2	18.6 ± 29.1	**0.007**
CRP (mg/L)	13.0 ± 11.0	9.2 ± 9.0	0.094
ESR (mm/h)	42.2 ± 22.0	54.5 ± 15.7	**0.005**
Plasma prohepcidin (ng/mL)	38.2 ± 39.3	17.9 ± 14.7	**0.004**

Measured variables were presented as mean±standard deviation. For statistical analysis, Kolmogorov-Smirnov test, Student's t-test, and Mann-Whitney U test were used

WBC: White blood cell, MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin; MCHC: Mean cell hemoglobin concentration, TIBC: Total iron binding capacity; CRP: C-reactive protein; ESR: Erythrocyte sedimentation rate

Discussion

Our findings revealed significantly higher serum iron concentration and ferritin levels along with higher serum prohepcidin levels in pregnancies complicated by preeclampsia, whereas no significant difference between preeclamptic and healthy pregnancy in terms of hematological parameters other than higher WBC and lower ESR count in the former group.

Our findings are in line with data from previous studies that indicated significantly higher serum iron concentration and ferritin levels in preeclamptic pregnancies than in normal pregnant women (5,6,8,9,12,13). Indeed, raised serum iron and ferritin levels are considered likely to be used diagnostically to detect incipient preeclampsia cases (5,12,13).

Inflammation-oxidative stress axis might perform a crucial role in the pathogenesis of numerous pregnancy complications, including preeclamsia (14-20). In high-risk pregnancies related to inflammatory conditions such as preeclampsia or obesity, hepcidin was reported to be increased compared to healthy pregnancies (8,10,21). Hence, the association of preeclampsia with an increase in BMI values in previous studies seems notable given the consideration of obesity as a risk factor of preeclampsia as well as the association of maternal obesity with increased hepcidin levels that leads to disturbed maternal-fetal iron transport (21,22). We included the control and preeclampsia patient groups that were matched for gestational age and BMI. As prohepcidin levels may vary with these factors, we eliminate them before introducing potential bias.

Moreover, our findings indicate that prohepcidin is detectable in the serum during the third trimester of pregnancy, and increased levels were associated with

preeclampsia, emphasizing the likelihood of prohepcidin to act like functional pro-hormone similar to active hepcidin. Notably, in the current study, increased prohepcidin levels among preeclamptic pregnant women were accompanied by an increase in serum iron levels, rather than a decrease, which is expected in the presence of elevated hepcidin via ferroportin internalization. This result seems to emphasize the likelihood of a presence of resistance to the iron-lowering effect of hepcidin in pregnancies complicated by preeclampsia. Hence, given the established role of iron overload in preeclampsia (8,12,13), our findings seem to indicate the likelihood of a vicious circle in terms of iron overload and concomitant hepcidin resistance, which may enhance the development of preeclampsia through ROS and inflammatory processes in the presence of an uncompensated increase in serum iron levels.

Likewise, increased hepcidin concentrations were reported to be along with increased plasma iron in preeclampsia, as suggested to be related to the inflammatory status due to increased ROS production. The authors also emphasized the presence of a hemochromatosis subtype with elevated plasma hepcidin concentrations, along with resistance to hepcidin due to structural or functional abnormalities of ferroportin (8).

Notably, in another study, based on significantly elevated levels of serum iron, ferritin, and hepcidin identified in preeclamptic pregnant women compared to controls, authors indicated the role of iron supplementation in the development of a state of iron overload and consequent oxidative stress and endothelial dysfunction in preeclampsia patients. The authors also reported that hepcidin with a cut-off level of 610 pg/mL differentiated preeclampsia from normal pregnancy and considered the rise in hepcidin levels in preeclampsia to act as a protective mechanism to counteract the iron overload mediated cytotoxicity (6).

Given that elevated iron levels in the presence of elevated hepcidin in preeclamptic pregnant women, our findings may also support the risk of iron overload in these women under iron supplementation as well as the associated increase in hepcidin levels to reduce ischemia-reperfusion injury and oxidative stress related to iron overload (6,23).

Accordingly, the exact role of elevated hepcidin coupled to increased serum iron levels in preeclamptic patients seems to be further investigated in terms of the likely presence of resistance to iron-lowering effect hepcidin or a protective mechanism to prevent the iron overload mediated oxidative stress, endothelial dysfunction, and cytotoxicity (6).

Nonetheless, it should be noted that there are also studies that reported an increase in serum iron and ferritin in preeclamptic pregnancies, whereas no significant difference between the preeclampsia and control groups in terms of prohepcidin levels (9) or lower hepcidin levels in preeclampsia (24). Also, no significant correlation was reported between prohepcidin level and iron status parameters in preeclamptic pregnant women (9,24). In contrast, the absence of correlation between iron homeostasis and hepcidin was considered to demonstrate the likelihood of factors specific for gestation to interact with hepcidin's iron decreasing action (8).

A significant positive correlation of hepcidin with CRP and interleukin-6 (IL-6) has been reported in previous studies (8,21,24,25). Likewise, preeclampsia was associated with elevated WBC count and a non-significant tendency for higher CRP values in the current study, emphasizing the role of inflammatory processes in increased hepcidin production and preeclampsia pathogenesis (10,26,27). Previous studies also revealed similar (8,12) or higher (28) values for hemoglobin and hematocrit between preeclamptic and normal pregnant women, as well as no significant difference between entire hematologic parameters (9).

Notably, in a systematic review of 16 studies, authors concluded that hepcidin is lower during pregnancy than in a non-pregnant status, with the lowest hepcidin concentrations detected in the third trimester, presumably to ensure greater iron bioavailability to the mother and fetus. Authors indicated that the inflammatory states, including obesity, infection, malaria, and preeclampsia, were related to higher hepcidin during gestation compared to healthy pregnant women, proposing that fetal and maternal iron bioavailability could be compromised in such circumstances (10). High third-trimester IL-6, ferritin, and hepcidin levels were also reported to be associated with higher neonatal complication risk (29).

Certain limitations to this study should be considered. First, due to the cross-sectional design, it is impossible to establish any cause-and-effect relationships. Second, the lack of data on blood findings during earlier pregnancy periods before the development of preeclampsia or subgroup analysis concerning the severity of preeclampsia is another limitation.

In conclusion, our findings indicate an increase in serum iron and ferritin levels coupled with elevated prohepcidin levels in pregnancies complicated by preeclampsia. Further longitudinal researches are needed to clarify the role of prohepcidin and active hepcidin in preeclampsia and the potential

mechanisms underlying the unregulated hepcidin-iron homeostasis.

Conflict of Interests: The authors declare that they have no conflict of interest.

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