Research Article

Prohepcidin in maternal circulation: is it related to spontaneous preterm labor?

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Background/aim: To investigate whether spontaneous preterm labor (PTL) with intact membranes is associated with changes in maternal serum prohepcidin concentrations.

Materials and methods: The study consisted of patients with spontaneous PTL with intact membranes (n = 25), a control group of healthy pregnant women between the 24th and 37th gestational weeks (n = 22), and uncomplicated term pregnancies in spontaneous labor (n = 19). Blood samples were collected from patients at the time of clinical diagnosis. Levels of prohepcidin, hemoglobin, serum ferritin, serum iron, unsaturated iron binding capacity, total iron binding capacity, transferrin and transferrin saturation, C reactive protein, and interleukin-6 were measured.

Results: Patients with spontaneous PTL had significantly lower maternal serum prohepcidin concentrations than term delivery and control subjects.

Conclusion: Maternal serum prohepcidin concentration is lower in patients with spontaneous PTL compared to term delivery and control subjects. This suggests that measuring maternal serum prohepcidin concentrations in PTL may be a feasible method for understanding etiologic causes of spontaneous preterm delivery, but, before suggesting this as a course of action, low levels of prohepcidin in patients with PTL need to be more fully investigated.

Key words: Spontaneous preterm labor, term pregnancy, prohepcidin

1. Introduction

The birth of a baby prior to 37 weeks of gestation is defined as a preterm birth and this complicates 5%–10% of all deliveries. Despite advances in medicine, the cause of preterm birth is in many situations elusive and unknown (1). Many factors appear to be associated with the etiology; there is overwhelming evidence that intrauterine infection and inflammation play an important role in the pathogenesis of spontaneous preterm labor (PTL). It is one of the major causes of perinatal mortality and morbidity, and a significant cost factor in healthcare. Early diagnosis of subclinical infection and inflammation may therefore aid clinicians in instigating interventions focusing on avoiding such adverse outcomes (2–5).

Hepcidin, a small peptide hormone produced by the liver, has a significant role in the regulation of iron homeostasis (6,7). It acts not only in iron regulation but also in host defense. Recently, hepcidin has been reported as a type 2 acute phase reactant. During infection and inflammation, hepcidin synthesis is markedly increased by a mechanism that is independent of erythropoietic activity (8–11).

Prohepcidin, another 84-amino acid peptide, is the precursor of the active hepcidin. Previous studies have shown that inflammatory stimulation of liver hepcidin synthesis is mediated by macrophage production of cytokines interleukin (IL)-6 and IL-1 in humans and mice (10,12). It has also been reported that serum prohepcidin levels were increased in neonatal sepsis as an acute phase antimicrobial peptide (13). Several studies have reported about the association between maternal inflammation and preterm deliveries. Different maternal serum and vaginal inflammatory markers such as C reactive protein (CRP), activin, inhibin, relaxin, and IL-6 were studied for predicting preterm births (14–17). These studies led us to consider that if serum prohepcidin is accepted as an acute phase reactant, the level of this peptide hormone might be changed in preterm delivery. Maternal prohepcidin concentration has been investigated in uncomplicated pregnancy, but the level of this peptide hormone in

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complicated pregnancies, such as cases of PTL, has not been investigated yet (18).

The aim of the present study is to investigate maternal serum prohepcidin levels in preterm pregnant women and to give new insight on the relation between maternal prohepcidin concentration and spontaneous PTL.

2. Materials and methods

2.1. Study design and population

This cross-sectional study was performed at the Turgut Özal University Obstetrics Unit in Ankara, Turkey, between September 2008 and September 2009. The study was approved by the ethics committee of the university and informed consent was obtained from the subjects.

This study included women in the following groups: patients with spontaneous PTL with intact membranes (n = 25), healthy pregnant women between the 24th and 37th gestational weeks as a control group (n = 22), and uncomplicated term pregnancies in spontaneous labor (n = 19).

Women were considered to have had an uncomplicated term pregnancy if they did not have any medical, obstetrical, or surgical complications and delivered a normal neonate at term (37–41 weeks of gestation) that was appropriately grown for the gestational age (19).

Spontaneous PTL was defined as the presence of regular uterine contractions occurring at a frequency of at least 2 every 10 min and associated with a cervical change that required hospitalization before 37 completed weeks of gestation.

The control group consisted of 22 healthy pregnant women from 24 to 37 gestation weeks who had no risk factors for preterm delivery and who underwent regular gynecological examinations.

The inclusion criteria for the women recruited for this study were: singleton pregnancy, hospitalization for PTL, hospitalization for spontaneous term labor, intact membranes.

Women with the following criteria were excluded: rupture of membranes, chorioamnionitis, fetal distress requiring extraction, fetal malformation, a maternal disorder requiring pregnancy termination, advanced labor (3 cm of cervical dilatation with uterine contractions).

All subjects had been given a daily supplement of 100 mg of iron as ferrous gluconate from 16 weeks of gestation until delivery. Microscopic examinations of cultures of urine and cervicovaginal secretions were carried out for all patients. Urinary infection and bacterial vaginosis were treated according to culture results.

Serum samples collected from all subjects for progesterone-induced blocking factor and cytokine determinations were aliquoted and kept at −80 °C until used. Blood samples were collected from preterm and

term patients at the time of clinical diagnosis. Each blood sample was analyzed for complete blood count, serum iron and ferritin concentrations, iron binding capacity, and prohepcidin level.

Levels of prohepcidin (ng/mL) were measured with an enzyme-linked immunosorbent assay (ELISA; DRG Instruments, Marburg, Germany). Hemoglobin (g/dL) was analyzed with a Beckman Coulter LH 750 hematology analyzer. Serum ferritin (ng/mL) was measured with an electrochemiluminescence assay (E170, Roche). Serum iron (µg/dL) and unsaturated iron binding capacity (UIBC) (µg/dL) were measured with a Roche Cobas Integra 800 autoanalyzer. Total iron binding capacity (TIBC) (µg/dL) values were calculated automatically from the sum of serum iron and UIBC, both of which were determined by colorimetric methods. Transferrin saturation was calculated directly as (Fe / TIBC) × 100 (%). Transferrin (mg/dL) and CRP (mg/L) were analyzed with a nephelometer (Beckman Coulter, IMMAGE immunoassay). IL-6 (pg/mL) was measured with a Siemens IMMULITE 2000 chemiluminescence immunoassay system.

2.2. Statistical analysis

Data analysis was performed using SPSS 11.5 for Windows (SPSS Inc., Chicago, IL, USA). Whether the continuous variables were normally distributed or not was determined by using the Shapiro–Wilk test. The Levene test was applied for the evaluation of the homogeneity of variances. Data are shown as mean ± standard deviation or median (minimum–maximum) as appropriate.

Means were compared by one-way ANOVA and the Kruskal–Wallis test was applied for the comparisons of the median values. When the P-value was statistically significant, post hoc Tukey or nonparametric multiple comparison tests were used to determine which groups differed from which others. Degrees of association between continuous variables were evaluated by either Pearson's product moment or Spearman's rank correlation test. A P-value of less than 0.05 was considered statistically significant.

3. Results

3.1. Clinical findings

There were no statistically significant differences between the groups of patients in terms of age, gravida, parity, or body mass index (BMI), but there was a statistically significant difference in terms of gestational age at delivery (P > 0.05) and birth weight of newborns (P > 0.05) (Table 1). Demographic and clinical characteristics of patients are listed in Table 1.

3.2. Maternal plasma prohepcidin levels

Maternal plasma prohepcidin levels were measurable in all samples evaluated. As shown in the Figure, maternal

Table 1. Demographic and clinical characteristics of women with term delivery, patients with PTL and intact membranes, and control group.

	PTL (n = 19)	Control (n = 22)	Term delivery (n = 25)	P
Aged (years)	27.8 ± 4.72	27.8 ± 3.59	27.9 ± 5.44	NS
Gravida	1 (1–3)	2 (1–4)	2 (1–4)	NS
Parity	1 (1–2)	1 (1–3)	2 (1–3	NS
Body mass index (kg/m²)	26.5 ± 1.81	27.2 ± 2.13	27 ± 1.48	NS
GA at delivery (weeks)	35.1 (25.4–36.5)	32.6 (27.4–36.5)	39.6 (37.3–41.4)	<0.05
Birth weight (g)	2440 (730–3610)	2310 (890–3450)	3420 (2930–3750)	< 0.05

plasma prohepcidin concentrations were significantly (P < 0.05) lower in women who delivered preterm [52.4 (39–242.3) ng/mL] than in controls [77.1 (36.3–149.1) ng/mL] and those who progressed to spontaneous term delivery [70 (34.4–116.7) ng/mL].

Serum Hb, iron, TIBC, transferrin saturation, and ferritin concentration were similar between groups. However, CRP, and IL-6 levels were higher in term delivery and PTL groups compared to the control group (Table 2).

No correlation was detected between maternal serum hepcidin concentration and maternal age, gravida, parity, gestational age, or BMI. Additionally, no correlation was detected between maternal serum hepcidin concentration and serum iron parameters in the groups.
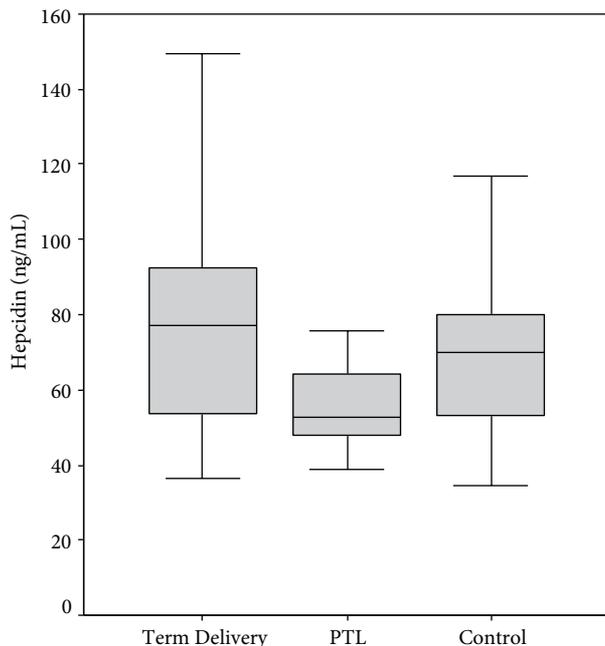
Figure. Maternal serum prohepcidin concentrations in normal term delivery, PTL, and control groups. Note that maternal serum prohepcidin concentration is elevated in both term delivery and control groups.

4. Discussion

The present study is the first trial to investigate maternal plasma prohepcidin concentration in women with preterm labor. The only limitation was the study size. According to our results, patients with spontaneous PTL had a significantly lower median maternal prohepcidin concentration than the term delivery patients and the control group. In addition, no correlation was detected between median maternal plasma prohepcidin concentration and iron parameters in the groups.

These results are not consistent with our expectations. When we designed this study, because prohepcidin has been shown to be a type II acute phase protein like other inflammatory markers, we thought it could be used to predict preterm labor. However, contrary to our expectations, maternal prohepcidin concentration was detected to be lower in PTL patients compared to term and control patients. This may be due to the iron-related effect of hepcidin. Iron is an essential element that is required for growth and development in all living organisms. At the same time, iron is dangerous to cells because of its ability to catalyze free radical generation. Oxidative damage caused by free radicals is thought to be causally related to numerous diseases, such as atherosclerosis, diabetes, cancer, and neurodegenerative diseases (20).

Different maternal iron parameters and their relationships with several obstetrical syndromes such as preeclampsia, intrauterine growth retardation, and PTL have been investigated in studies. Iron deficiency anemia in pregnancy is a known risk factor for preterm delivery (21).

Iron transport increases significantly during pregnancy due to the high growth rates of the fetus and placenta and the expansion of the maternal red blood cell mass. The availability of iron for the fetus depends on maternal iron stores and intestinal absorption of iron (22). Transport of iron via the placenta is usually sufficient to meet the needs of the fetus, except in cases of severe iron deficiency (23). There is a little evidence that hepcidin is also involved in transplacental iron transfer, but its role in this process is

Table 2. Serum concentrations of maternal iron parameters and acute phase reactants (mean ± SD).

	PTL	Control	Term delivery	P
Hepcidin (ng/mL)	52.4 (39–242.3)	77.1 (36.3–149.6)	70 (34.4–116.7)	
Hemoglobin (g/dL)	12 ± 1	11.9 ± 1.04	11.1 ± 3.38	NS
Serum iron (µg/dL)	53 (27–470)	68 (25–539)	56 (28–214)	NS
TIBC (µg/dL)	528 ± 66.94	472 ± 85.92	504.3 ± 65.04	NS
Serum ferritin (ng/mL)	18 (5.8–54)	17.5 (8–47)	20 (7.3–44)	NS
Transferrin (mg/dL)	402 (300–543)	348.5 (252–539)	457.5 (339–5334)	<0.05
Transferrin saturation	13 (6.5–34)	15 (6–31)	13.1 (7.9–18.3)	NS
CRP (mg/L)	6.8 (2–93)	4.7 (2–16)	9 (1–76)	<0.05
IL-6 (pg/mL)	2.7 (1–92)	1 (1–61)	3.6 (1–181)	<0.05

not fully understood. The general role of prohepcidin is to be a negative regulator of iron export via ferroportin and therefore to prevent the iron efflux from cells (24).

Hepcidin is mainly secreted by the liver, but smaller amounts of hepcidin mRNA are also expressed in other organs, e.g., the heart, brain, and lungs, and also in the placenta (25,26). Generally, iron carried by transferrin in maternal serum is bound to the transferrin receptor on the placental cells, and both are endocytosed into the placental cells. Iron transferred through the placental cells is then mediated through ferroportin to the fetal circulation (27). The amount of ferroportin in the cell membrane is regulated by hepcidin (28).

Maternal prohepcidin levels have been studied in normal pregnancy (18), but the level of plasma prohepcidin has not been investigated before in spontaneous PTL. In preterm delivery, prohepcidin levels have been detected in maternal serum and decreased levels of maternal prohepcidin may adversely influence iron homeostasis in the placenta and cause an iron overload in the placenta. The placental burden of iron can be a reason for hypoxia-induced preterm delivery. In mice models, hepcidin gene expression is decreased in anemia and in hypoxic conditions (11).

Hereditary hemochromatosis is the most well-known disease related to hepcidin. In hereditary hemochromatosis, dietary iron absorption is increased above the amount required to replace losses, and excess iron is deposited in the liver, endocrine glands, heart, and skin (29).

Eventually, the iron-overloaded organs suffer tissue damage, presumably through the iron-catalyzed generation of reactive oxygen species. A similar relationship may be true for spontaneous PTL, e.g., acquired maternal prohepcidin insufficiency causes iron overload in the placenta and that causes damage to the placenta.

Hepcidin expression decreases progressively throughout pregnancy, reaching its lowest level just before

parturition, and then approaches normal levels soon after birth. Decreases in the level of hepcidin probably trigger PTL. Even so, it is not known what the normal level of maternal serum prohepcidin is in the third trimester; we compared maternal prohepcidin levels and detected low levels of maternal serum prohepcidin in the spontaneous PTL group. However, we are not sure that these low levels of maternal serum prohepcidin are a reason for, or a consequence of, spontaneous PTL.

Some studies have reported significant correlations between maternal serum prohepcidin concentration and serum iron status measurements (30,31), but there are also studies showing opposite findings (32,33). We also did not show any correlation between maternal serum prohepcidin levels and iron parameters in the groups. However, maternal serum IL-6 and CRP levels were detected to be increased in both term and PTL patients compared to the control group. Similarly, maternal serum iron binding capacity and transferrin levels were detected as higher in both term and PTL patients compared to the control group. This is probably related to the effect of labor on inflammatory markers.

Prohepcidin is an inactive prohormone from which active hepcidin is cleaved (18). Reliable immunoassays for hepcidin have been recently reported but require further validation before their use can be generalized (25,34). Thus, many studies have used prohepcidin ELISA measurement in iron homeostasis studies (35).

In conclusion, our results show that inappropriately low maternal serum prohepcidin levels in pregnant women may be the cause of iron-mediated tissue damage through placental iron loading leading to subclinical fetal hypoxia, which could trigger PTL. Whatever the role of the decreased levels of circulating maternal prohepcidin concentrations, the clinical usefulness of maternal serum prohepcidin measurement for predicting the occurrence of PTL merits further consideration.

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