Cervicovaginal Biomarkers and C-reactive Protein Levels in Preterm and Term Labor

Massome Rezaei[1], Shole Shahgheibi[2*], Roonak Shahoei[3], Farnaz Zadvakili[1], Fariba Farhadifar[2], Narjes Noori[4], Fariba Saiedalshoiadaei[1]

[1]Assistant Professor OBGYN Ward, Kurdistan University of Medical Sciences, Sanandaj, Iran
[2*]Associated Professor OBGYN Ward, Kurdistan University of Medical Sciences, Sanandaj, Iran
[3]Assistant Professor, Midwifery Department, Kurdistan university of Medical Sciences
[4] OBGYN Ward, Zahedan University of Medical Sciences, Zahedan, Iran
*Corresponding Author: shahgheibi@yahoo.com

Abstract: Objective: To assess the levels of cervicovaginal biomarkers (HCG and FFN) and serum CRP in primigravida women who terminated their gestation with term labor versus those who delivered prematurely. **Design and setting:** In this nested cohort study, 89 patients with symptoms of labor were enrolled in Beasat Hospital in Sanandaj, Capital in Kurdistan province, in Iran. **Methods:** Data regarding the patients' age, education, BMI, and hemoglobin level were recorded. Laboratory test results including the levels of CRP, FFN, and HCG were also recorded and patients were monitored until the end of gestation to obtain the ultimate pregnancy result (preterm/term). The mean difference between groups was determined using the *t* test. **Results:** 43 patients were 21-24 years old, 53.93% patients had a BMI of 20-24, and 63.87% patients had term labor. 49.43% were CRP negative . 26 and 80 patients were HCG negative and FFN positive respectively. FFN and CRP were the only markers that were significantly higher in patients with preterm labor (P<0.001). **Conclusion:** FFN appears to be the strongest predictor of preterm birth followed by CRP, whereas HCG assay may not render a reliable prediction of preterm birth.

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Introduction

Preterm labor, defined as delivery prior to the completion of 37 weeks gestation, is currently known as one of the major causes of prenatal mortality and morbidity and accounts for 60-80% of deaths due to otherwise congenital defects (1). Despite recent advances in the early diagnosis of this condition, about 10-50% of women presenting with signs and symptoms of preterm delivery actually deliver prematurely (2). Numerous factors such as premature rupture of the amniotic membrane, preeclampsia, myoma, cervical defects, and fetal disorders have been mentioned as potential etiological factors for preterm labor, however, cervical infection and inflammatory mediators are likely to be the most important factors (3-5). The inflammatory response to the colonization of bacterial pathogens on the decidua and extraplacental membranes and the release of cytokines, in particular IL-1, leads to an elevation in the prostaglandins level and subsequent preterm delivery (3, 6, and 7). Diagnosis of preterm labor has always been a challenge for clinicians. C-reactive protein (CRP) is an acute phase reactant protein, which is produced in response to inflammation and remains stable in normal conditions. Elevated levels of this marker have been associated with intrauterine inflammation (8, 9). Fetal fibronectin (FFN) and human chorionic gonadotropin hormone (HCG) have also been used as markers to predict preterm labor. FFN is an extracellular glycoprotein, produced by trophoblasts, which is localized at the maternal-fetal interface of the amniotic membranes, between the chorion and decidua. Elevated levels of FFN greater than 50 mg/mL at or after 22 weeks in cervicovaginal secretions are a strong predictor of preterm birth (10). HCG is a glycoprotein hormone, which interacts with luteinizing hormone/choriogonadotropin receptors and promotes the maintenance of corpus luteum as a major source of progesterone (2, 11-13). Recent studies have indicated that there is significant correlation between high levels of IL-1, 6, and 8 in the cervicovaginal fluid and preterm labor (14, 15). We aimed to assess the levels of cervicovaginal biomarkers (HCG and FFN) and serum CRP in primigravida women who terminated their gestation with term labor versus those who delivered prematurely in an Iranian population in 2008.

Material and methods

In this nested cohort study, 89 primigravida women referring to Be'asat Hospital, Sanandaj, west of Iran in 2008 were included. Among these women, 29 had a gestational age of 24-36 weeks and premature labor pain (uterine contractions every 5-8 minutes with a minimum of 1 cm cervical dilatation),

and 60 women with term pregnancy and labor pain. Healthy 18-40 year-old women with normal pregnancy (without the mediation of drugs) and no history of systemic conditions and cervical malformations, BMI between 18-26, absence of bleeding, intact amniotic sac, and nulliparity were included. Symptoms of preterm delivery were initially confirmed in clinical examination by a senior resident of gynecology and obstetrics. Informed consent was obtained from all patients prior to recruitment. Multiparity, enlarged uterus because of hydrous amnious, chorioamnionitis, abnormal fetus, antibiotic consumption up to one week before the study, and smoking were considered as exclusion criteria. Information on maternal demographic (age, weight, last menstrual period, education) and medical characteristics (preterm delivery symptoms, laboratory test results and final pregnancy result) was recorded in a structured questionnaire.

To determine the levels of FFN and HCG, samples from the cervicovaginal secretions were obtained using an endocervical swab and transferred to the immunology laboratory of Sanandaj Univeristy of Medical Sciences. Blood samples were also obtained to measure the CRP concentration in both the preterm (case) and the term (control) labor group. The cytokines and the CRP levels were measured using ELISA and Immuno-turbidimetry with the Olympus AU S60 system, respectively. Data were analyzed using SPSS software, version 15, and subjected to analysis using the *t* test.

Results:

Table 1 presents the mean age, hemoglobin, CRP, FFN, and HCG among patients in the term and preterm delivery groups. 43 (48.31%) patients were 21-24 years old, 27 (30.33%) were 17-20 years old, 13 (14.60%) were 25-28 years old and only 6 (6.74%) patients were 29-32 years old. The mean Hb level and BMI among the study population was 12.5±51.20 and 25.32±3.59, respectively. Of the 29 patients in the case group, 18 patients (20.22%) terminated their pregnancy with term labor and 11 (12.35%) delivered prematurely. 18 patients with symptom of preterm labor continued their pregnancy to term. 44 (49.43%) patients were CRP negative (<10 mg/l). 35 (39.32%) patients had serum CRP level in the range of 11-20 mg/l, 6 (6.74%) patients in the range of 21-30 and 4 (4.49%) patients had a range of 31-40 mg/l. The overall mean concentration of serum CRP was 12.97±8.52 mg/l among the study population. 26 (29.21%) and 80 (89.88%) patients were HCG negative (<2 mIU/ml) and FFN positive (>50 ng/ml), respectively. Statistical analysis revealed that CRP and FFN were significantly higher in the preterm delivery group (P<0.001) [Table 2]. Furthermore, Levene's test for homogenity of variance demonstrated significant difference in CRP (P=0.008) and FFN (P=0.01) levels between the preterm and term delivery groups [Table 3].

Table 1- Demographic and medical characteristics of patients in the preterm and term delivery groups (Mean±SD)

Group	Age	Hb	CRP	HCG	FFN
Term delivery group	22.66±2.65	12.73±0.97	7.99±3.56	5.59±5.02	13.39±7.29
Preterm delivery group	22.28±3.71	12.34±1.43	18.13±9.13	10.60±19.60	25.49±13.29

Table 2- *T* test results for the comparison of study variables in the preterm and term delivery groups

Variables	Mean difference	Significance
HB	0.39	0.24
CRP	10.13	0.000*
FFN	12.09	0.000*
HCG	5.01	0.18
BMI	0.30	0.75

Table 3- Levene's test results assessing the homogeneity of variance among the study variables in the preterm and term delivery groups

Variables	F	Significance
HB	0.40	0.53
CRP	8.25	0.008*
FFN	7.14	0.01*
HCG	2.28	0.14
BMI	3.06	0.09
Age	0.215	0.64
Weight	0.392	0.53
Height	0.586	0.45

Table 4: *T* test results for the comparison of study variables with 95% confidence interval		
Variables	Mean difference	Significance
HB	0.33	0.59
CRP	3.19	0.36
FFN	-13.03	0.008*
HCG	8.25	0.27
BMI	-0.73	0.653

Table 4 provides the *t* test results and the mean differences in Hb, CRP, HCG, and FFN among the patients in the preterm group who actually ended up in term delivery versus those who delivered prematurely.

Discussion

This was a prospective cohort study, which compared the levels of CRP, HCG, and FFN among patients who underwent preterm delivery versus those who underwent term delivery. Our analysis revealed that the cervico-vaginal fibronectin and serum CRP levels were significantly higher in the preterm delivery group (P<0.001). We further observed that in the preterm-delivery group, the only biomarker which was significantly higher in patients who actually delivered prematurely was FFN. This suggests the significant role of FFN in the prediction of premature delivery (10).CRP is an acute-phase reactive protein, which is produced in response to the release of IL-6 and TNF α (16, 10). BMI and the general adiposity can affect the concentration of serum CRP (17, 18). Several studies have documented an association between increased levels of CRP and the risk of preterm birth. Lohsoonthorn and colleagues demonstrated that the early elevation of CRP concentrations (≥7.5 mg/l) is a strong predictor of preterm delivery (19). Hvilsom and colleagues (2002) and Pitiphat and coworkers (2005) have also reported similar results (20, 21). Our findings, however, failed to support Borna's study in which CRP was not deemed a significant marker in the prediction of preterm birth (22).

In our study, FFN assay was shown to be the most reliable tool in the early diagnosis of preterm labor. Ruiz and coworkers (2006) reported that the relative risk of preterm delivery was 2.22 (CI: 1.09, 4.55, P<0.015) when FFN was found to be positive at any weekly testing after 22 to 24 weeks of gestation (76.82% sensitivity, 58.33% specificity) (23). In Wilms study, the fibronectin test had a sensitivity and specificity of 92% and 60%, respectively, with a positive predictive value of 27% and negative predictive value of 98% (24). A Cochrane systematic review evaluated the efficacy of this marker in 474 pregnant women and concluded that although FFN is commonly used in delivery units, there is not sufficient evidence to recommend its use (10). We failed to demonstrate a significant correlation between elevated levels of cervicovaginal HCG and the risk of preterm delivery. Adhikari (12), Garshasbi (13), and Gurbuz (25), however, reported cervicovaginal HCG assay as an effective tool for the prediction of preterm birth. In Adhikari's study, HCG levels ≥45 mIU/ml was referred to as a diagnostic marker with sensitivity and specificity values of 95.8% and 73.7%, respectively. The cut-off point in Garshasbi's study was 77.8 mIU/ml and HCG levels greater than 77.8 mIU/ml between 20 and 28 weeks' gestation, led to the diagnosis of preterm birth with a sensitivity of 87.5% (95% CI: 47.4-97.9) and a specificity of 97% (95% CI: 86.5-99.4) with positive and negative predictive values of 88.5% and 98%, respectively. Gurbuz and colleagues have concluded that compared with fibronectin assay, HCG was a less expensive test with more availability and could render accurate results in the prediction of preterm labor (12, 13, and 25).

Conclusion

Cervicovaginal fibronectin appears to be the strongest marker in the prediction of preterm birth and maternal CRP could also be used as a valuable tool for early diagnosis of this condition.

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Corresponding Author:
Shole Shahgheibi, Obstetrics and Gynecology Ward, Besat Hospital, Keshavarz Street, Sanandaj, Iran,
Email: shahgheibi@yahoo.com
Shole.shahgheibi@muk.ac.ir

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