Cervical Mucus SLPI is a Predictive Biomarker for Timing of Delivery in Nulliparous Term Pregnancies with Unfavorable Cervix

Keywords
Secretory leukocyte protease inhibitor, SLPI, prediction of delivery time, unfavorable cervix

Abstract

Introduction
The aim of the present study was to evaluate cervical mucus secretory leukocyte protease inhibitor (SLPI) levels for the prediction of delivery time in uncomplicated term nulliparous pregnancies with an unfavorable cervix.

Material and methods
In this prospective study, a total of 88 nulliparous singleton pregnant women with a gestational age between 370/7and 376/7 weeks were included. Bishop Score was determined and unfavorable cervix was defined as a Bishop Score <5. Cervical mucus SLPI levels were collected from pregnant women with an unfavorable cervix between 370/7and 376/7gestational weeks. Patients were divided into two groups according to gestational age at delivery after cervical mucus SLPI sampling: delivery within 2 weeks group (n: 48) and delivery after 2 weeks group (n: 40).

Results
Maternal demographic characteristics, mean Bishop Score, transvaginally measured cervical length, and cervical dilatation at cervical mucus SLPI sampling were similar between the two groups. The mean cervical mucus SLPI level was 52.4±52.6 ng/mL in the delivery after 2 weeks group and 253.2±220.0 ng/mL in the delivery within 2 weeks group and statistically different. The optimal SLPI cutoff value was 32 ng/mL for predict labor induction requirement due to absence of spontaneous labor before 410/7 (sensitivity, 0.65; specificity, 0.93; positive predictive value, 0.52; negative predictive value, 0.95).

Conclusions
Our results suggest that measuring the SLPI levels in the cervical mucus can be a useful biomarker for predicting spontaneous delivery time in uncomplicated term nulliparous pregnancies with an unfavorable cervix.

Cervical Mucus SLPI is a Predictive Biomarker for Timing of Delivery in Nulliparous Term Pregnancies with Unfavorable Cervix

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Materials and Methods: In this prospective study, a total of 88 nulliparous singleton pregnant women with a gestational age between $37^{0/7}$ and $37^{6/7}$ weeks were included. Bishop Score was determined and unfavorable cervix was defined as a Bishop Score <5. Cervical mucus SLPI levels were collected from pregnant women with an unfavorable cervix between $37^{0/7}$ and $37^{6/7}$ gestational weeks. Patients were divided into two groups according to gestational age at delivery after cervical mucus SLPI sampling: delivery within 2 weeks group (n: 48) and delivery after 2 weeks group (n: 40).

Results: Maternal demographic characteristics, mean Bishop Score, transvaginally measured cervical length, and cervical dilatation at cervical mucus SLPI sampling were similar between the two groups. The mean cervical mucus SLPI level was 52.4±52.6 ng/mL in the delivery after 2 weeks group and 253.2±220.0 ng/mL in the delivery within 2 weeks group and statistically different. The optimal SLPI cutoff value was 32 ng/mL for predict labor induction requirement due to absence of spontaneous labor before $41^{0/7}$ (sensitivity, 0.65; specificity, 0.93; positive predictive value, 0.52; negative predictive value, 0.95).

Conclusion: Our results suggest that measuring the SLPI levels in the cervical mucus can be a useful biomarker for predicting spontaneous delivery time in uncomplicated term nulliparous pregnancies with an unfavorable cervix.

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Introduction

The remodeling of the cervix is an important step in the beginning of labor. Cervical ripening involves reorganizing the extracellular matrix and occurs before labor in a term pregnancy [1]. Cervical dilation occurs after the tissue is rearranged into a soft and flexible structure. There is increasing evidence suggesting that the physiological control mechanism for the initiation of labor in the uterus is the infiltration of immune cells containing neutrophils and activated macrophages [2]. Cervical remodeling is the rearrangement of the extracellular matrix in cervical tissue before labor, which then transforms into a soft and stretchable structure [3] and begins to dilate. Increasing evidence has shown that cervical regeneration is subject to precise regulation by the immunological mediators and proteases produced [2].

Secretory leukocyte protease inhibitor (SLPI) is a low molecular–weight secretory protein found in the alveolar face of the lung, intestinal mucosa, and mucosal surface of the reproductive system [4]. The antimicrobial, anti-inflammatory, and antiprotease functions of SLPI have been demonstrated and they provide antiprotease activity with several types of serine proteases, including neutrophil elastase, cathepsin G, and trypsin [5]. Because of SLPI's anti-inflammatory and antiprotease effects, it protects tissues from excessive inflammation and helps maintain tissue homeostasis [6]. Itaoka et al. have suggested that SLPI levels in the cervical mucus are significantly higher in preterm deliveries than in term deliveries. These results support the role of SLPI in pregnancy maintenance and the regulation of cervical regeneration, such as the onset of labor [7]. In routine clinical practice, despite the intense efforts to determine the molecular mechanisms responsible for delivery, it remains difficult to predict this onset. Therefore, in the present study we aimed to evaluate the role of cervical mucus SLPI levels on the timing of labor in nulliparous term pregnancies.

Materials and Method

The Erciyes University Ethics Committee approved this prospective study (approval no: 2019/389). The study occurred at the Kayseri City Education and Research Hospital Turkey. The Declaration of Helsinki was followed and all participants gave their written informed consent before participating. Overall, 120 consecutive nulliparous pregnant women who had a singleton cephalic presentation fetus and gestational age between $37^{0/7}$and $37^{6/7}$ weeks were included. In the presence of multiple gestations, known major fetal anomalies, pre-gestational or gestational hypertension, preeclampsia, pre-gestational or gestational diabetes, preterm rupture of membranes, placenta previa, placenta invasion anomalies, presence of active genital herpes infection, or human immunodeficiency patients were excluded from the study. In addition, patients were excluded if there was an active cervicovaginal infection or areas of cervical ulceration and erosion that may affect SLPI levels. Cervical mucus SLPI levels were collected from pregnant women with an unfavorable cervix between $37^{0/7}$and $37^{6/7}$gestational weeks. Before collecting samples, cervical dilatation, effacement, station, cervical position, and cervical consistency were evaluated and the Bishop score was determined. An unfavorable cervix was defined as a Bishop score <5.

Pregnant women were followed up to $41^{0/7}$ weeks of gestation with a weekly non-stress test, amniotic fluid measurement, and biophysical profile. As a routine practice of our clinic, labor induction was preferred at $41^{0/7}$ gestational weeks. During this follow-up, 32 pregnant women who needed an emergency delivery for obstetric reasons, an emergency Cesarean section, or required labor induction for any obstetric reason were excluded from the study. The study included 88 pregnant women in the final analysis. Gestational age at delivery was used to divide patients into two groups; within two weeks (n: 48) and after two weeks (n: 40) (Figure 1).

The cervical mucus was sampled as previously described [4]. The samples were obtained with the women in the lithotomy position prior other vaginal maneuvers. A sterile cotton-tipped swab was used on the outer cervical ostium area for 10 seconds to remove the cervical mucus samples, which were then dipped in 1 mL phosphate buffered saline solution and stirred. To minimize any process-related variability in the samples, a single obstetrician (MES) gathered the samples using a similar cotton-tipped sterile swab for each sampling. The eluted samples were centrifuged at 10000 rpm at 4°C for 10 min, after which the supernatant was placed in Eppendorf tubes and stored at -80°C until analyses. SLPI concentrations were analyzed using the enzyme-linked immunosorbent analysis method (Cusabio-E09308h kit catalog number). Bradford method used for measuring the total protein concentration in each sample. The crude concentration values of SLPI were normalized to the total protein concentrations.

Statistical analyses

An analysis of variance was used to compare the groups followed by data analyses using Minitab 16 (Minitab Inc., State College, PA, USA).The normality of the data was determined using the Shapiro–Wilk test. The assumption of equal variance was assessed using Levene's test. The values are expressed as the mean ± standard deviation. The Student's *t*-test was used for parametric comparisons and the Mann–Whitney U test was used for nonparametric comparisons. The chi-squared test was used to evaluate n% values. The statistical significance level was $p < 0.05$.

Results

Table 1 illustrates a comparison of the maternal and fetal characteristic sat the time of cervical mucus SLPI sampling. Maternal age (p=0.260), BMI>30 kg/m^2 (p=0.510), ethnicity (p=0.830), education levels (p=0.680), and smoking (p=0.730) were similar between the two groups. Gestational age at SLPI sampling (p=0.960), biparietal diameter (p=0.690), head

circumference (p=0.840), abdominal circumference (p=0.980), and femur length (p=0.810) were similar between the two groups. The mean Bishop score at cervical mucus SLPI sampling (p=0.570), transvaginally measured cervical length (p=0.280), and cervical dilatation (p=0.340) were similar between the two groups.

Table 2 provides delivery characteristics and cervical mucus SLPI levels between the two groups. Mean gestational age at delivery, spontaneous vaginal delivery, and cesarean requirement at delivery were significantly different between the two groups (p<0.001). Birth weight (p=0.740), male newborn rates (p=0.930), and 5 min Apgar score (p=0.850) were similar between the two groups. The mean cervical mucus SLPI level was 52.4±52.6 ng/mL in the delivery after 2 weeks group and 253.2±220.0 ng/mL in the delivery within 2 weeks group and statistically different (p<0.001). ROC curve analysis showed that optimal SLPI cutoff value was 32 ng/mL for predict labor induction requirement due to absence of spontaneous labor before $41^{0/7}$ (sensitivity, 0.65; specificity, 0.93; positive predictive value, 0.52; negative predictive value, 0.95).

Discussion

When there is a risk to both the fetus and mother of continuing the pregnancy, labor is induced; however, this process is also associated with nearly double the number of cesarean section births, especially in nulliparous women with unfavorable cervixes [8]. In fact, there is a positive linear relationship between the increase in the practice of inducing delivery and rising national Cesarean section rates [9]. In the current study, we aimed to evaluate cervical mucus SLPI levels for delivery prediction time in uncomplicated term nulliparous pregnancies. The key findings are: (1) The mean cervical mucus SLPI level was 52.4±52.6 ng/mL in the delivery after 2 weeks group and 253.2±220.0 ng/mL in the delivery within 2 weeks group and elevated cervical mucus SLPI levels were significantly related to delivery time within 2 weeks; (2) ROC curve analysis showed that optimal SLPI cutoff value was 32

ng/mL for predict labor induction requirement due to absence of spontaneous labor before $41^{0/7}$ (sensitivity, 0.65; specificity, 0.93; positive predictive value, 0.52; negative predictive value, 0.95); (3) Measuring the SLPI levels in the cervical mucus can be a useful biomarker for predicting spontaneous delivery time in uncomplicated term nulliparous pregnancies with an unfavorable cervix.

As mentioned, cervical ripening progresses before delivery in term pregnancies [3, 10], after which the cervix beings to dilate. Increasing evidence suggests that these alterations of the cervix are regulated by local immunologic mediators and proteases [2]; however, studies that have evaluated these local immunologic mediators and proteases associated with delivery are limited. Itaoka et al. have demonstrated that expression levels of SLPI messenger RNA in cervical cells increase after the first trimester, which suggests an association between SLPI levels and the hormonal environment. As peripheral blood progesterone concentrations increase during pregnancy, their high levels at term may, in part, contribute to SLPI levels [11]. In a study with uncomplicated term pregnancies, Samejima et al. prospectively obtained cervical mucus samples weekly until delivery at routine prenatal visits beginning at >37 weeks of gestation. They found that SLPI levels collected within 7 d of delivery are significantly higher than those collected >7 d before delivery. The ROC curve analysis reported that the cutoff value for the SLPI to total protein ratio for predicting delivery within 7 d was 1.62 mg/ml (sensitivity, 0.69; specificity, 0.72). Therefore, SLPI might be a candidate biomarker for delivery time prediction [12].

We suggest our results can be explained using the conditions of the pre-delivery cervical microenvironment. Activated leukocytes, including macrophages and neutrophils, produce SLPI [13]. Subsets of these leukocytes infiltrate the cervical tissue during pregnancy [14]. Hence, the increased cervical mucus SLPI levels observed here may indicate the immune cell increases in the cervix prior to delivery. The morphologic conditions of the

peripartum uterine cervix tissue drastically change from a closed rigid structure that keeps the growing fetus from emerging from the uterus into dilated malleable tissue that allows passage through the pelvic canal. These important changes are initiated by pro-inflammatory mediators and proteases [2]. Even more remarkable is that the cervix returns to its original structure after delivery with no significant tissue damage or fibrosis. Considering the unique multifunctional molecular features of SLPI (i.e., anti-inflammatory, antiprotease, and antimicrobial properties), the roles of its enhanced expression near the end of pregnancy are protect the cervical tissue damage that might result from excessive inflammation and facilitate cervical recovery. Evidence suggests that SLPI plays a pivotal role in these activities [15].

Growing Cesarean rates in Turkey are of concern, and these procedures are higher among women for which labor is induced. The American College of Obstetricians and Gynecologists' Practice Bulletin on labor induction advises that nulliparous women with unfavorable cervices for which labor is induced should be provided information on the two-fold higher risk of morbidity and mortality to both them and their newborns of Cesarean delivery after induced delivery [16, 17]. Therefore, it remains challenging to predict which patients will have positive results from labor induction. SLPI levels in cervical mucus appear to be an important biomarker that provides new horizons in this area. Secondly, attempting vaginal birth (VBAC) after having a Cesarean section has become increasingly important in obstetrics. Prelabor nomograms are used to predict its success [18, 19]. In particular, if VBAC is attempted in patients who have undergone a previous Cesarean section because of failed labor or unsuccessful labor induction, evaluation of SLPI levels in the cervical mucus before induction would be suggested for induction success. Adding these levels as a biomarker to the known prelabor nomograms in these patients will further increase this predictive value.

The strengths of this study include its large sample size and the inclusion of uncomplicated term nulliparous pregnancies in a tertiary university hospital. Exclusion of

factors that may affect cervical mucus SLPI levels and factors that may affect the success of labor induction showed the cervical remodeling process and the effect of SLPI more clearly. We are aware that there are limitations of the study. Using a single marker for labor induction success is one of them.

Conclusion

Our results suggest that measuring the SLPI levels in the cervical mucus can be a useful biomarker for predicting spontaneous delivery time in uncomplicated term nulliparous pregnancies with an unfavorable cervix.

Disclosure

All authors have declared that they have no conflicts of interest associated with this study.

Data Availability

The data used to support the findings of this study are available on request from the corresponding author.

Authors' contributions

MES, ES; Study design, contributed substantially to the study concept and design and the acquisition, analysis, and interpretation of data

YM, ICM, MMK; provided clinical interpretation of the results, wrote the first draft of the manuscript

CK; contributed to biochemical analyses

MES; very critically revised the manuscript.

All authors gave approval for the publication of the final version

Ethical approve

This study was approved by the Erciyes University Ethics Committee (approval no: 2019/389).

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Figure 1. Patient flow chart

Table 1. Comparison of the maternal and fetal characteristics at cervical mucus SLPI sampling.

Table 2. Comparison of the delivery characteristics and cervical mucus SLPI levels between groups

Table 1. Comparison of the maternal and fetal characteristics at cervical mucus SLPI sampling.

	Delivery within 2 weeks group (n = 48)	**Delivery after 2 weeks group (n = 40)**	**p-value**
Maternal age (years)	27.7 ± 3.0	27.2 ± 2.4	0.260
BMI>30 kg/m^2 (n[%])	5 (10.4)	6 (15)	0.510
Ethnicity (Caucasian) (n [%])	40 (83.3)	34 (85)	0.830
Education (high school graduate) (n [%])	17 (35.4)	13 (32.3)	0.680
Smoking (n [%])	6 (12.5)	4 (10)	0.730
Gestational age at SLPI sampling (weeks)	37 (37–37)	37(37–37)	0.960
BPD (mm)	86.2 ± 4.1	88.9 ± 6.2	0.690
HC (mm)	332.6 ±26.4	334.4 ±28.2	0.840
AC (mm)	314.3 ± 20.4	318.6 ± 23.4	0.980
FL (mm)	72.4 ± 4.8	74.8 ± 5.3	0.810
Bishop score at cervical mucus SLPI sampling	2 (1-2)	2 (2-3)	0.570
Cervical length (transvaginal ultrasound) (mm)	34.3 ± 4.3	36.4 ± 5.8	0.280
Cervical dilatation			

(transvaginal ultrasound) (cm)	0.7 ± 0.2	0.9 ± 0.2	0.340

Notes: SLPI, secretory leukocyte protease inhibitor; BMI, body mass index; BPD, biparietaldiameter; HC, head circumstance; AC, abdominal circumstance; FL, femur length

Table 2. Comparison of the delivery characteristics and cervical mucus SLPI levels between groups

	Delivery within 2 weeks group (n = 48)	**Delivery after 2 weeks group (n = 40)**	**p-value**
Gestational age at delivery (weeks)	38 (38–39)	40(40–41)	<0.001
Cervical mucus SLPI levels (ng/mL)	253.2 ± 220.0	52.4 ± 52.6	<0.001
Mode of delivery (spontaneous vaginal) (n [%])	44 (91.6)	31 (77.5)	<0.001
Cesarean requirement at delivery (n [%])	4 (8.3)	9 (22.5)	<0.001
Birth weight (g)	3180 ± 250	3320±270	0.740
Male newborn (n [%])	28 (58.3)	23 (57.5)	0.930
5 min Apgar score	9(8-10)	9 (9-10)	0.850

Notes: SLPI, secretory leukocyte protease inhibitor;

Figure 1. Patient flow chart