OBSTETRICS

Elevated concentration of secretory leukocyte protease inhibitor in the cervical mucus before delivery

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BACKGROUND: Cervical remodeling during parturition progresses under exquisite regulation by immunologic mediators and proteases. Secretory leukocyte protease inhibitor is a secretory protein that can function as an antimicrobial peptide, an antiinflammatory molecule, and a protease inhibitor. The involvement of secretory leukocyte protease inhibitor in cervical remodeling before and during parturition is understood poorly.

OBJECTIVE: We aimed to reveal the role of secretory leukocyte protease inhibitor in the cervical remodeling process before normal term delivery and to evaluate its utility as a predictive biomarker for timing of delivery.

STUDY DESIGN: Cervical mucus samples were collected prospectively at weekly prenatal visits from a cohort of pregnant women at term. The secretory leukocyte protease inhibitor concentrations in 95 mucus samples that were obtained from 49 women with uncomplicated pregnancy who subsequently underwent normal vaginal delivery were assessed. Alterations in secretory leukocyte protease inhibitor concentrations at term and the association of secretory leukocyte protease inhibitor levels with the time to delivery were analyzed.

RESULTS: A moderate positive correlation with significance was detected between cervical mucus secretory leukocyte protease inhibitor concentrations and days to delivery ($r = 0.38$; $P = .0001$). The secretory leukocyte protease inhibitor concentration was significantly higher in samples that were collected within 7 days of delivery when compared with samples that were collected >7 days before delivery ($P = .001$). Secretory leukocyte protease inhibitor concentrations were also significantly higher in samples from women with premature rupture of membranes when compared with those without premature rupture of membranes ($P = .01$), all of whom delivered within 7 days. A logistic regression analysis revealed that the cervical secretory leukocyte protease inhibitor level was a significant parameter for the prediction of the onset of delivery. ($P = .017$; unit odds ratio, 1.28; 95% confidence interval, 1.07–1.61). A cut-off value of cervical secretory leukocyte protease inhibitor/total protein to predict delivery within 7 days was determined to be 1.62 μg/mg (sensitivity, 0.69; specificity, 0.72) using receiver operating characteristic curve—analysis.

CONCLUSION: Secretory leukocyte protease inhibitor concentrations in the cervical mucus elevate progressively before delivery in uncomplicated term pregnancies. Our findings suggest that cervical secretory leukocyte protease inhibitor is a candidate biomarker for delivery prediction.

Key words: antiprotease, biomarker, delivery, inflammation, prediction, secretory leukocyte protease inhibitor, term pregnancy

Cervical remodeling is an essential step in the initiation of parturition. Cervical ripening is a reorganization of extracellular matrix structure that progresses before labor at term pregnancy.[1,2] After this alteration of tissue property into soft and extensible structure, cervical dilation occurs. To date, a growing body of evidence suggests that cervical remodeling is under exquisite regulation by locally produced immunologic mediators and proteases.[3] The concept that parturition is a physiologically controlled inflammatory event is well-acknowledged and demonstrated by an infiltration and accumulation of hematopoietic immune cell that includes neutrophils and activated macrophage in the uterus on the onset of labor.[3] The growing body of literature suggests that an activation of proinflammatory factors begins at the stage of cervical dilation rather than cervical ripening.[4-6] Microarray analysis of the cervical transcriptome in term pregnant women revealed that cervical dilation in spontaneous labor is characterized by the overexpression of the genes that are related to neutrophil chemotaxis and extracellular matrix regulation.[4]

Even though protracted and intense efforts have outlined a general understanding of molecular mechanisms that are responsible for parturition in humans, precise prediction of labor onset remains a daily challenge in modern obstetrics. Molecular markers that are detectable in biologic samples that include amniotic fluid, cervicovaginal fluid, and cervical mucus have been tested for their utility not only in prediction of the date of delivery in normal pregnancies but also in assessment of the risk of preterm labor.[7] So far, their clinical utility has been limited because of unsatisfactory performance in terms of sensitivity and specificity.

Secretory leukocyte protease inhibitor (SLPI) is a low-molecular-weight secretory protein that is expressed at the mucosal surfaces of a variety of tissues that include the alveoli of the lung, the intestine, and the reproductive tracts.[8] SLPI is a member of the whey acidic protein family, member of which are characterized by 4-disulfide core domains rich in cysteine residues.[9] SLPI has several biologic functions and can act as

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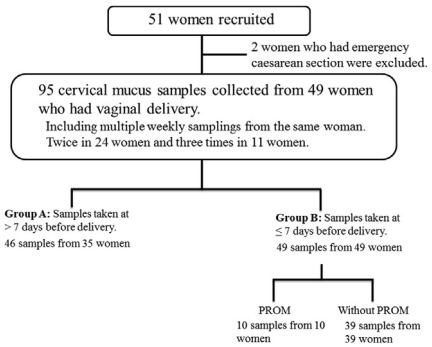
FIGURE 1
Study design and description of cohort



Fifty-one women at term pregnancy without complications were recruited. After the exclusion of 2 women who went through emergency cesarean delivery, 95 samples were obtained from 49 women who had spontaneous vaginal delivery. For analysis, cervical mucus samples were divided into two groups retrospectively. Group A included those samples that were obtained at >7days before delivery and group B included those samples that were obtained at ≤7 days before delivery. Group B was subcategorized depending on the incidence of preterm rupture of membranes.

PROM, preterm rupture of membranes.

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an antimicrobial peptide, an antiinflammatory molecule, and a protease inhibitor.[10] The antiprotease activities of SLPI cover several kinds of serine proteases that include neutrophil elastase, cathepsin G, and trypsin. SLPI expression is enhanced at sites of inflammation in response to lipopolysaccharide and proinflammatory cytokines.[11,12] Considering its antiinflammatory and protease inhibitory properties, SLPI is thought to play a critical role in protection against tissue damage on excessive inflammation and maintenance of tissue homeostasis.[13]

Constitutive expression of SLPI within the female genital tract has been described.[14-17] SLPI concentrations in cervical mucus are under the influence of the menstrual cycle, with highest levels detected in the ovulatory phase.[15] In human pregnancy, SLPI protein expression has been confirmed in the cervical mucus plug and fetal membranes.[16] We previously have reported that SLPI expression levels in cervical cells increased over the course of human pregnancy.[17] Additionally, cervical SLPI expression during pregnancy was significantly higher in women who ultimately had a preterm birth compared with control women who delivered at term.[17] These findings suggested a role for SLPI in the maintenance of pregnancy and its involvement in the regulation of cervical remodeling as parturition approaches.

In this study, we primarily aimed to clarify the association between cervical mucus SLPI concentrations and the cervical remodeling process before delivery and prospectively to investigate alterations in SLPI concentration in a cohort of women who attended routine prenatal care near the end of their pregnancies. Additionally, we evaluated the utility of SLPI as a predictive marker for time to delivery.

Material & Methods

Study design and target samples

This study was conducted under the approval of the institutional review board of the University of Tokyo and Showa General Hospital. Consent was given before clinical sample collection. Cervical mucus samples were obtained in a prospective manner, weekly until delivery, at routine prenatal visits that occurred at >37 weeks of gestation. Women with known obstetric complications (such as preeclampsia, gestational diabetes mellitus, and threatened preterm labor) were excluded. Women with symptoms of rupture of membranes or vaginal bleeding at sampling were excluded. Only women who subsequently delivered vaginally after spontaneous onset of labor with or without premature rupture of membranes were included. After the recruitment, 2 women were excluded from this study because of emergency cesarean delivery: a woman who showed nonreassuring fetal status on a cardiotocogram before labor onset and a woman pregnancy ended in induction failure after mechanical dilation of the cervix at postterm period.

Forty-nine women with term pregnancies without major obstetric and nonobstetric complications were included in the analyzed study population. In 38 women, sampling was performed at >1 weekly intervals before delivery; sampling was performed twice in 27 women and 3 times in 11 women. Ultimately, 95 cervical mucus samples from 49 women were used for the analyses (Figure 1).

Cervical mucus sampling and clinical data collection

During vaginal speculum examination, the end of a cotton-tipped swab was placed into the external cervical os for 10 seconds to obtain distal cervical mucus. All the cervical mucus sampling was conducted by a single experienced obstetrician to minimize interobserver variability. The swab was stirred immediately in 1 mL of phosphate-buffered saline solution that contained 0.25 mol/L NaCl to dissolve the mucus. The eluted sample was centrifuged at 15,000 rpm at 4°C for 10 minutes, and the supernatant was stored at −80°C until batched protein measurement.

Clinical data were collected by a review of the medical records of enrolled women after delivery.

Measurement of SLPI concentration

SLPI concentrations were determined by enzyme-linked immunosorbent assay (ELISA), with the use of the Human SLPI Quantikine ELISA kit (Assay range, 62.5−4000 pg/mL; R&D systems, Minneapolis, MN). The total protein concentration in each sample was measured with the Bradford method (Bio-Rad protein assay, BIO-RAD, Tokyo, Japan). The crude concentration values of SLPI were normalized to total protein concentrations.

Statistics

Statistical analysis was conducted with JMP Pro software (version 11; SAS Institute Inc, Cary, NC). Differences in background characteristics between sample groups were evaluated with *t*-testing for parametric data and