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Original Article

Neutrophil elastase in cervical fluid in women with short cervical length☆

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ABSTRACT

Objective: The aim of the study was to determine the relationships between short cervical length (CL) and levels of cervical fluid neutrophil elastase (NE), secretory leukocyte protease inhibitor (SLPI), and interleukin 8 (IL-8) in the second trimester of pregnancy of women who underwent ultrasound-indicated cervical cerclage.

Materials and methods: CL of <25 mm or cervical funneling were included in the short CL group (n = 26) and the normal CL group (n = 22) included women who had CL of ≥25 mm and had no cervical funneling in women between 17 + 0 and 24 + 6 weeks of gestation. Levels of NE, SLPI, and IL-8 were measured by using enzyme-linked immunosorbent assay kits. Mann–Whitney *U* tests and Spearman's correlation analysis were used for statistical analyses.

Results: Compared with the normal CL group, the short CL group had significantly higher median NE levels ($P < 0.001$) and higher, though not significant, median IL-8 levels by approximately three times (2107.0 vs. 798.3 pg/mL, $P = 0.132$). The median SLPI levels in cervical fluid was similar between the two groups (107.6 vs. 103.2 ng/mL, $P = 0.499$). Short CL had a significant correlations with cervical fluid NE levels ($r = -0.475$, $P = 0.001$).

Conclusion: Increased cervical fluid NE associated with cervical shortening in second trimester of pregnancy, whereas cervical fluid SLPI had constant levels.

Introduction

The uterine cervix provides mechanical strength and immunological functions to modulate infection and inflammation during pregnancy. Shortening of the uterine CL in the second trimester was demonstrated to be a strong predictor of spontaneous preterm delivery [1]. Women with shortened CL in the second trimester of pregnancy need to receive cervical cerclage and vaginal progesterone administration in order to reduce the risk of preterm birth and improve perinatal outcomes [2].

However, the distinct mechanisms responsible for cervical shortening in the second trimester remain unknown and incompletely characterized. One study investigated the relationship between the expressions of vaginal proinflammatory cytokines and cervical shortening in women at high risk of spontaneous preterm labor and those who underwent cerclage and vaginal progesterone treatment [3]. They reported that women who had short CL (<25 mm) before 24 weeks of gestation exhibited higher cervicovaginal concentrations about only two among 11-plex fluid-phase immunoassay, granulocyte-macrophage colony-stimulating factor (GM-CSF) and monocyte chemotactic protein 1 (MCP-1). So they showed limited relationship between cervico-vaginal fluid cytokine profiles and cervical shortening. Women with increased cervical mucus IL-8 levels and who underwent cerclage because of cervical shortening had increased rate of preterm delivery [4]. Some studies performed on women just undergoing preterm labor have analyzed the relationship between cervical or cervico-vaginal inflammatory cytokines and preterm birth. In preterm labor, cervico-vaginal fluid interleukin-6 (IL-6) and IL-8 were predictors

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of impending preterm delivery [5]. And, an increased level of granulocyte elastase in cervical secretions was an independent predictor for preterm delivery before 34 weeks of gestation [6]. In addition, there was a study about preterm delivery and anti-inflammatory cytokines, SLPI and elafin. Cervical mRNA of SLPI was significantly higher in patients who experienced preterm labor and delivered before term than in those who delivered at term [7]. And IL-8 is a potent neutrophil chemotactic factor and recruited neutrophil release NE [8,9]. SLPI is known as anti-inflammatory, anti-infectious, and has anti-protease properties about as like NE [10].

The mechanisms responsible for the accumulation of granulocytes in the ripe cervix have not been clarified yet. During human cervical ripening, macrophages and neutrophils, which are prominent sources of proteases, collagenases, and elastase, infiltrate the cervix. These enzymes digest extracellular matrix (ECM) components in the uterine cervix, including collagen, elastin, and proteoglycan [11]. In particular, elastase depolymerizes and degrades collagen in the ripening cervix [12]. After collagen bundles disperse and lose strength, cytokines, elastase, and collagenases possibly work together to allow effacement [13].

In this study, we investigated the levels of cervical fluid NE, SLPI, and IL-8 in women with shortened CL (<25 mm) in the second trimester of pregnancy who underwent ultrasound-indicated cervical cerclage and their relationships to short CL.

Materials and methods

Participants

This prospective study was conducted in 48 consecutive women with singleton pregnancies between 17 + 0 and 24 + 6 weeks of gestation. The women who were referred with short CL (<25 mm) or cervical funneling were included in the short CL group (n = 26). The normal CL group included women who had CL of ≥25 mm and had no cervical funneling (n = 22) noted on routine prenatal check-up. Among the women with short CL, three had no funneling but had CL of 4.1, 8.5, and 14.9 mm, respectively. One woman had a prior preterm birth at 20 weeks of gestation. None of the women in the normal CL group had experienced a previous preterm delivery. The exclusion criteria included multiple pregnancies, preterm premature membrane ruptures, major fetal anomalies, any gross cervical bleeding, clinical evidence of chorioamnionitis [14], and other medical or surgical problems.

The Institutional Review Board for Clinical Research of the Hallym University Kangnam Sacred Heart Hospital approved this study (No. 2013-08-71), and informed consent was obtained from all the participants.

Procedures

After informed consent, women in the short CL group were managed with McDonald cervical cerclage or vaginal progesterone administration. Before the cerclage procedure at the operating room, cervical fluid samples were collected. After cerclage, CL was measured again 2–3 days later. Another informed consent was obtained from the women who were undergoing routine prenatal checkup, cervical fluid samples were collected and transvaginal CL was measured the normal CL group. Women who had foul vaginal odor, greenish vaginal discharge, curd-like vaginal discharge, and gross cervicitis on sterile speculum examination were excluded. CL was measured by residents and a well-trained technician using an ultrasonographic machine equipped with a 5-MHz transvaginal probe (General Electric, Fairfield, CT, USA) [15]. In brief, after emptying the bladder, the vaginal probe was gently placed in the anterior fornix of the vagina to obtain a sagittal view of the complete cervix, including the internal os, external os, and endocervical canal. The probe was slowly withdrawn until the image blurred, and the insertion pressure was increased just enough to restore the clear cervical image. CL was measured by placing the electronic markers at the furthest points between the internal os and external os, measured as a straight line. The shortest of the three measurements obtained was recorded as the CL.

Samples were obtained by placing a sterile cotton-tipped swab into the endocervical canal filled with cervical mucus through the external cervical os and twirling it for 10 s to aspirate 100 μL of cervical fluid, which was immediately transferred to a sample buffer solution (1% bovine serum albumin, 0.5 mM phenylmethylsulfonyl fluoride, 0.5 unit of aprotinin as trypsin inhibitor, or 0.1 μg/mL leupeptin in Tris-buffered saline [pH 7.4]), stirred for 5 s, and left to stand for 30 min at room temperature. Debris and cells were removed by centrifugation at 1500 g for 15 min, and the supernatants were stored at −70 °C until used for analysis. SLPI and IL-8 levels in cervical fluid were measured in duplicate by using commercially available enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, Minneapolis, MN, USA). The kit sensitivities for SLPI and IL-8 were 25 pg/mL and 7.5 pg/mL, respectively. NE level was also measured in duplicate by using an ELISA kit specific for NE (NOVUS, Littleton, CO, USA), with 1.98 pg/mL sensitivity. The intra-assay and inter-assay variabilities of the three kits were between 4% and 10%.

Statistical analysis

Statistical analysis was performed by using IBM SPSS Statistics version 19.0 for Windows (IBM Corp., Armonk, NY, USA). The results are presented as medians and ranges. The Mann–Whitney *U* test was used to determine the significance of the differences between the short and normal CL groups. Spearman's correlation analysis was used to investigate possible correlations between CL; cervical fluid SLPI, NE, and IL-8 levels; cervical funneling depth; gestational age at delivery; preterm delivery (<34 weeks of gestation); and maternal serum C-reactive protein (CRP) levels. A P value of <0.05 was considered statistically significant.

Results

The clinical and demographic characteristics of the study subjects are compared in Table 1. At the time of the study, maternal age, preterm delivery history, parity (>1), and gestational age were not significantly different between the short and normal CL groups. However, the median CL was 15.0 mm in the short CL group and 33.3 mm in the normal CL group ($P < 0.001$). In addition, the women in the short CL group delivered earlier than those in the normal CL group (36.0 vs. 39.4 weeks of gestation, $P = 0.004$), with seven women delivering at <34 weeks of gestation. In the short CL group, the median depth of cervical funneling was 16.5 mm (range, 0.4–22.1 mm); serum CRP levels, 4.3 mg/L (1.6–6.4 mg/L); CL after cerclage, 27.4 mm (21.6–32.6 mm); and funneling depth after cerclage, 0.6 mm (0.3–1.4 mm).

The median NE levels were significantly higher in the short CL group (177.9 vs. 89.2 ng/mL, $P < 0.001$), but the median SLPI levels in cervical fluid were similar between the two groups (107.6 vs. 103.2 ng/mL, $P = 0.499$). The SLPI/NE ratio was significantly lower (0.63 vs. 1.24, $P = 0.001$) in the short CL group. In addition, the median IL-8 levels in the short CL group were higher by about three times that in the normal CL group, but the difference was not significant (2107.0 vs. 798.3 pg/mL, $P = 0.132$) (Table 2).

Significant correlations were found between CL and cervical fluid NE levels (Spearman's rho coefficient: $r = -0.475$, $P = 0.001$)