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CLINICAL STUDY

Pro-inflammatory mediators in vaginal fluid and short cervical length in pregnancy

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ABSTRACT

AIM: We hypothesized that elevated vaginal levels of matrix metalloproteinase-8 (MMP-8), interleukin-8 (IL-8) and the 70kDa heat shock protein (hsp70), compounds involved in inflammatory responses, correlated with a short cervix in pregnant women.

METHODS: This prospective cohort study used a convenience sample of 64 women in their early third trimester with a singleton pregnancy. A short cervical length was present in 35 women (54.7 %). Vaginal fluid was tested for levels of MMP-8, IL-8 and hsp70 by enzyme-linked immunosorbent assay (ELISA). A receiver operating charasteristic (ROC) analysis was used to calculate the area under the curve (AUC) for each mediator in predicting short cervical length.

RESULTS: MMP-8 (109 vs 29.6 ng/ml, p=0.014), IL-8 (689 vs 330 pg/ml, p=0.007) and hsp70 (4.4 vs 2.9 ng/ml, p=0.036) were all elevated in vaginal samples from women with a short cervix. In addition, there was a negative association between the concentration of each compound in vaginal fluid and cervical length p≤0.026). The vaginal IL-8 concentration had the highest negative correlation with a short cervix (AUC=0.7, p=0.007).

CONCLUSION: MMP-8, hsp70 and IL-8 contribute to a pro-inflammatory cervico-vaginal milieu that weakens cervical integrity and leads to a shortening in cervical length *(Tab. 4, Fig. 1, Ref. 27)*. Text in PDF *www.elis.sk.*

KEY WORDS: cervical length; heat shock protein; interleukin-8; matrix metalloproteinase-8; vaginal fluid.

Introduction

A short cervix in pregnant women, usually defined as a cervical length (CL) < 25 mm, is a well-known risk factor for subsequent premature delivery. It is one of the most cost-effective methods in current clinical practice to predict risk for preterm birth (1). Many variables have been associated with cervical shortening including genetic factors, trauma, uterine stretching, infection or cervical hemorrhage (2, 3).

There remains a need to determine the mechanism(s) responsible for a shortened CL in individual women so that the most appropriate counter-measures can be initiated. In this communication we focus on the measurement of three compounds in vaginal fluid that plausibly are involved in inflammation-related cervical shortening in some women.

Matrix metalloproteinase (MMP)-8, also known as neutrophil collagenase, plays a major role in reorganizing extracellular matrix tissue. Its presence is necessary for degrading the extracellular matrix and basement membrane (4). Interleukin-8 (IL-8) is a chemokine that targets neutrophils to promote their chemotaxis and activation as well as induce reactive oxygen species (ROS) (5). Its presence in cervico-vaginal fluid has been associated with cervical dilation, preterm labor, labor at term, cervico-vaginal infection, amniotic fluid infection, chorioamnionitis and adverse cerclage outcome (6–11). The inducible 70kDa heat shock protein (HSPA1A), commonly known as hsp70, is a protein produced in response of stress. When released from cells it acts as a potent activator of pro-inflammatory immunity (12).

The present study evaluated in women with singleton pregnancies possible associations between vaginal levels of MMP-8, IL-8 and hsp70 and CL. Our aim was to determine if these compounds could serve as potential biomarkers for the presence of inflammation-related cervical alterations.

Materials and methods

This was a prospective cohort study using a convenience sample of women in their third trimester with a singleton pregnancy seen for routine prenatal care at Careggi Hospital in Florence, Italy, between 2014 and 2018. Exclusion criteria were multifetal gestations, the presence of uterine or fetal malformations, signs or symptoms of a genital tract infection, antibiotic usage in the previous 2 weeks or inability to provide informed written consent. The final study group consisted of 35 women with a CL < 25 mm

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and 29 women whose cervix was ≥ 25 mm. For ethical reasons, all women with a short CL were prescribed vaginal progesterone, 100 mg twice a day, from time of detection (after vaginal sample collection), until delivery. Age, parity, gravidity, CL and gestational age at time of sample collection and at delivery were recorded. All subjects self-reported as White.

CL was measured via transvaginal ultrasound by experienced personnel. Each woman emptied her bladder prior to the procedure. A vaginal probe was introduced into the vagina and positioned to visualize the endocervical canal. The image was enlarged and centered to fit the monitoring screen. Calipers were placed at the internal and external os and the distance between them was measured.

Blood was obtained by venipuncture and after clot formation the serum fraction was collected by centrifugation. Vaginal secretions were collected with a swab and then placed in phosphate buffered saline (PBS). The soluble fraction was obtained by centrifugation and stored in aliquots at –80 °C before testing.

Vaginal levels of MMP-8, IL-8, hsp70 were quantitated using a commercial multiplex assay. Values were converted to ng/ml or pg/ml by reference to a standard curve that was generated in parallel to each assay.

Normality of data was evaluated by the Shapiro-Wilk test. Categorical variables were compared with the Chi-square test and continuous variables with the Mann–Whitney test. Correlation between non-parametric distributed variables was analyzed with the Spearman rank correlation test. Receiver operator characteristic (ROC) analysis was used to confirm the association between levels of each mediator and CL, comparing the different areas under the curve (AUC). A $p < 0.05$ was considered significant. The statistical analysis was performed with SPSS (IBM, Chicago, US).

Results

Subjects' characteristics are shown in Table 1. The two groups did not differ in age, gestational age at sample collection or delivery, or history of preterm births. Five women from each group delivered preterm. Both gravidity and parity were higher in women with a normal CL ($p \leq 0.02$).

Table 2 compares the vaginal levels of MMP-8, hsp70 and IL-8 in both subject groups. MMP-8 (109 vs 29.6 ng/ml, p=0.014), IL-8 (688.5 vs 329.8 pg/ml, p=0.007) and hsp70 (4.2 vs 2,9 ng/ml p=0.036) were each higher in women with a short cervix than in controls. In addition, the vaginal level of IL-8 was strongly correlated with levels of MMP-8 and hsp70 (p<0.001). There was no association between number of prior gestations and levels of IL-8, MMP-8 or hsp70.

Vaginal concentrations of MMP-8 (p=0.026), hsp70 (p=0.001) and IL-8 (p=0.010) were each negatively associated with CL (Tab. 3). Vaginal IL-8 had the strongest negative correlation with CL (AUC 0.7, p=0.007) (Tab. 4, Fig. 1).

Tab. 1. Demographics and pregnancy outcomes in study subjects.

Variable	Short cervical length (n=35)	Normal cervical length (n=29)	p
Age (years)	34 (29–37)	35 (28–39)	NS
Gravidity > 0	14 (40%)	20 (68%)	0.021
Parity > 0	5 (14%)	11 (37%)	0.03
GA at collection (weeks)	29 (27–31)	28 (26–30)	NS
Cervical length at specimen collection (mm)	16 (10–21)	30 (27–35)	<0.001
GA at delivery (weeks)	38 (37–40)	39 (37–39)	NS
Preterm delivery	5 (14%)	5 (12%)	NS

Short cervical length is defined as a cervix <25 mm. GA – gestational age; NS – not significant. Values – except for percentages, are given as median (range)

Tab. 2. Vaginal fluid concentrations of MMP-8, hsp70 and IL-8 in the study subjects.

Mediator	Short cervical length (n=35)	Normal cervical length (n=29)	p
MMP-8 (ng/ml)	109 (31–210)	29.6 (4.5–81.3)	0.014
IL-8 (pg/ml)	688.5 (420.7–1234.9)	329.8 (85.5–868.18)	0.007
Hsp70 (ng/ml)	4.2 (2.9–22.4)	2.9 (2.3–6.6)	0.036

Values are given as median (range).

Tab. 3. Correlations between mediator concentration and cervical length.

Mediator	Correlation coefficient	p
MMP-8	–0.281	0.026
IL-8	–0.325	0.010
Hsp70	–0.364	0.004

Tab. 4. ROC (receiver operating characteristic) curve analysis of association between mediators in vaginal fluid and cervical length.

Mediator	AUC	p
MMP-8	0.680	0.014
IL-8	0.700	0.007
Hsp70	0.654	0.041

AUC – area under the curve

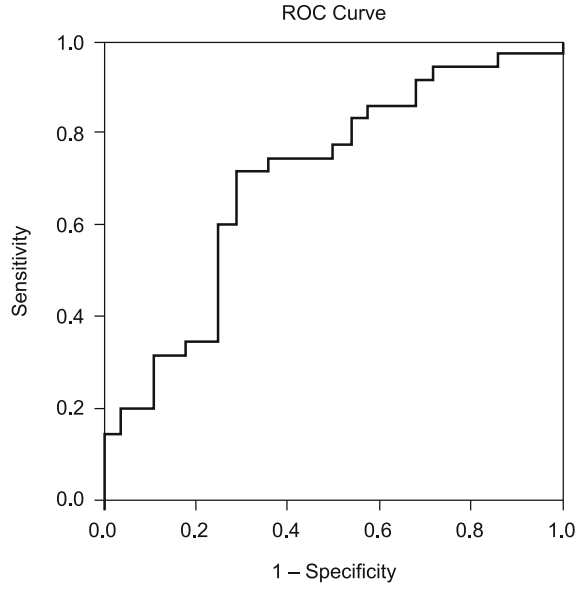

Fig. 1. ROC (receiver operating characteristic) curve analysis of vaginal IL-8 association with short cervical length. Area Under the Curve (AUC) 0.7. p = 0.007.

Discussion

We demonstrated a strong association between cervico-vaginal levels of MMP-8, IL-8 and hsp70 and detection of a CL < 25 mm in pregnant women in their early third trimester. Furthermore, IL-8 concentrations were highly correlated to levels of both MMP-8 and hsp70. These observations support a role for these compounds in contributing to a local pro-inflammatory immune milieu that weakens cervical integrity and results in a shortening of the cervix.

Levels of IL-8 normally decrease throughout gestation and elevated cervico-vaginal IL-8 concentrations have been associated with spontaneous preterm birth. IL-8 influences the transcription of MMPs. Endothelial cells incubated with IL-8 were shown to have increased expression of MMP mRNA (13). IL-8-induced activation of MMPs also occurs as a mechanism to promote cell proliferation (14). MMP-8 is an enzyme that promotes breakdown of the extracellular matrix in a variety of physiological and pathological processes. Its primary function is the degradation of collagen to achieve tissue remodeling (15). MMP-8 may also have a reciprocal role in IL-8 activation. An analysis of MMP-8 in breast cancer cell lines revealed a causal connection between MMP-8 activity and IL-8 production (16). Synthesis of hsp70 is greatly up-regulated when cells encounter physiological stress. It is released into the extracellular milieu and activates pro-inflammatory immunity to counter the perceived threat (17). Hsp70 is also an MMP inducer. It has been shown to activate MMP and facilitate the migration and invasion of malignant breast cells (18). Hsp70 induction of MMP gene expression occurs via activation of the transcription factors, NF-κB and AP-1 (19). These factors simultaneously promote the transcription of genes coding for multiple pro-inflammatory cytokines (20, 21). NF-κB and AP-1 up-regulate the transcription of IL-8 in cervical epithelial cells (22).

An association between IL-8 levels in cervico-vaginal fluid and short CL has been observed previously (11) and elevated serum levels of both MMP-8 and IL-8 have been associated with preterm delivery (10). Conversely, two earlier studies did not find associations between vaginal MMP-8 levels and short CL or preterm delivery (11, 23). Differences in study populations, sample collection and storage and assay sensitivity may account for these differences. Extracellular hsp70 has previously been reported to be elevated in sera, umbilical cord and placenta of women who delivered preterm (24, 25). Increased vaginal levels of hsp70 have also been associated with bacterial vaginosis (BV) in mid-trimester pregnant women (26). Pregnant women with BV are known to be at increased risk for a preterm birth (27).

Limitations of the study include the small sample size and the absence of inclusion of a protocol to ascertain vaginal bacterial composition. As noted above, women whose cervix was ≥ 25 mm had a higher gravidity and parity than women with a cervix <25 mm. However, pregnancy history did not influence vaginal levels of IL-8, MMP-8 and/or hsp70. Pregnancy outcome was not a variable that could be analyzed in the present study since all women with a short cervix received prophylactic treatment.

MMP-8, IL-8 and hsp70 are biomarkers as well as likely participants in the mechanism leading to a premature shortening of CL in pregnant women that increases susceptibility to preterm labor and delivery. Larger studies are needed to assess the clinical benefit of measuring these compounds concomitantly with CL evaluation to better assess the risks of preterm delivery.

Learning points

- MP-8, IL-8 and hsp70 are associated with a short cervical length during pregnancy.
- IL-8 concentrations were highly correlated to levels of both MMP-8 and hsp70.

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