Matrix metalloproteinase-1 and -9 in cervicovaginal fluid from women during pregnancy and in labor

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Abstract

Objective. We sought to determine whether cervicovaginal fluid matrix metalloproteinase-1 and -9 (MMP-1 and MMP-9) levels differed during pregnancy compared with those at term or in preterm labor.

Study design. We used sensitive immunoassays to measure MMP-1 and -9 levels in cervicovaginal secretions. Cases (n=32) included women who delivered preterm, and were sampled more than 3 week prior to delivery (n=19), within 1 week of delivery (n=7) and during spontaneous labor (n=6). Controls consisted of 80 women matched for race, age and gestational age, delivering at term and who were sampled at 20-32 weeks (n=47), within one week of delivery (n=14) and during term labor (n=19).

Results. Among cases and controls, cervicovaginal MMP-1 levels were low and unaffected by labor. Among non-laboring control patients the median and range of MMP-9 concentrations were also low (0; 0-0.04 ng/ml), and these remained unchanged with advancing gestational age. However, MMP-9 levels increased significantly within 1 week of term labor (0.8; 0-22.8 ng/ml; p=0.001), and during term labor (6.6; 0-30.6 ng/ml; p=0.04) with the highest values observed among laboring patients with ruptured membranes (24.8; 19.2-30.6 ng/ml; p=0.002). Among cases, MMP-9 concentrations were unaltered prior to preterm labor, but increased among patients in preterm labor (0.3; 0-30 ng/ml; p=0.02).

Conclusion. Cervicovaginal MMP-1 levels were low and unchanged during either preterm or term labor. In contrast, MMP-9 levels increased during term and preterm labor but did not predict preterm delivery in asymptomatic patients.
Introduction

The delivery of infants prior to 37 weeks of gestation complicates 10% of births in the USA\(^1\) and is a leading cause of neonatal morbidity and mortality.\(^2\) Traditional methods of predicting women at risk that rely on obstetrical history, demographic factors or premonitory symptoms are inaccurate.\(^3\) Biochemical and biophysical techniques that have been employed for prediction of preterm delivery include intermittent antenatal uterine contraction monitoring,\(^4\) transvaginal sonographic measurement of cervical length,\(^5-8\) assays of biochemical markers such as salivary estriol,\(^9,10\) corticotrophin-releasing hormone,\(^11-13\) cytokines and fetal fibronectin in cervicovaginal secretions.\(^14,15\) With the exception of fetal fibronectin, none of these endpoints alone, has proved sufficiently sensitive and specific to justify its incorporation into routine antenatal care at low or even high risk.

We postulated that the release of chorionic fetal fibronectin and structural changes in the cervix preceding labor reflect proteolytic degradation. The human cervix is predominantly a fibrous organ with less than 15% smooth muscle. It has a highly organized arrangement of collagen fiber bundles in a proteoglycan matrix, which gives it the structural rigidity it needs to retain the conceptus until term.\(^16\) Matrix metalloproteinases (MMPs) are a family of zinc-dependent extracellular matrix-degrading enzymes. Interstitial collagenase (MMP-1) cleaves collagen types I, II and III.\(^17\) The gelatinases (MMP-2 and -9) further degrade denatured collagen fragments generated by interstitial collagenase. MMPs and their natural inhibitors are produced by the amnion, chorion and decidua and are important in the maintenance and breakdown of the extracellular matrix of the amniochorion and cervix.\(^17\) The presence of MMP-1 has been studied in human cervical tissue,\(^18\) the placenta,\(^19\) amniotic fluid,\(^20\) and serum.\(^21-24\) Those studies provide evidence that MMP-1 plays an important role during cervical dilatation in human parturition. The involvement of MMP-9 in parturition has been demonstrated in the rat amnion,\(^25\) human amniochorion,\(^26\) amniotic fluid,\(^27,28\) urine\(^29\) and plasma.\(^30\) The purpose of our study was to evaluate whether MMP-1 and -9 are detectable in the cervicovaginal secretions of women during pregnancy and in preterm and term labor, and, if so, whether cervicovaginal MMP-1 and/or -9 may be useful markers of preterm birth.

We hypothesized that, since the MMP’s act to regulate the fetal membrane and extracellular matrix of the cervix, there should be a measurable elevation in their
concentrations in cervicovaginal fluid during the process of breakdown of fetal membrane structure and the process of cervical ripening. Further, increased concentrations should be apparent whether these processes are occurring at term or preterm.

Materials and Methods

Data reported in this study were acquired as part of an ongoing prospective cohort study to evaluate sociodemographic, biochemical and clinical factors that may be used for the prediction of preterm delivery. Study sites included the Women’s Health Clinic at Bellevue Hospital Center, New York, USA and the Department of Obstetrics and Gynecology of the University Medical Center Utrecht, the Netherlands. The local institutional review boards approved the study and we obtained informed consent from all patients. Term delivery was defined as delivery at or after 37 completed week’s gestation. All patients had an estimated date of confinement determined by the date of last menstrual period (LMP) and confirmed by serial ultrasounds.

Cases (n=32) included women who delivered preterm, and where sampled more than 3 weeks prior to delivery (n=19), within 1 week of delivery (n=7) and during spontaneous labor (n=6). Controls consisted of 80 women matched for race, age and gestational age, delivering at term and who were sampled at 20-32 weeks (n=47), within 1 week of delivery (n=14) and during labor (n=19). All participants screened negatively for bacterial vaginosis and sexually transmitted diseases.

The collection of cervicovaginal secretions during pregnancy was performed sequentially at 3-4 week intervals between 20 and 32 weeks’ gestation. For this cross-sectional study, only one specimen for each gestational age (20-24, 25-28, 29-32 weeks) was used for analysis. All comparison groups contained one sample only for each woman. In the event that we had more than one sample available for analysis, we selected the sample from the earliest gestational age period. Spontaneous preterm and term labor were defined as active labor resulting in delivery with cervix dilatation. Vaginal and cervical samples were collected separately from the posterior fornix of the vagina and from the endocervical canal during a speculum exam using a Dacron swab. Since we detected no difference between MMP-1 or MMP-9 concentrations collected from the vagina or the cervix, we report values for vaginal specimens only, since these were collected
more frequently. The swab was placed in an antiprotease solution, containing an anti-protease buffer consisting of 750 µL of 1% bovine serum albumin in Tris buffer (BSA-TBS) with 5 mmol/l EDTA, 5 mmol/l phenolmethylsulfonylfloride (PMSF) and 0.5 trypsin inhibitory units of aprotinin. This buffer does not effect the MMP-1 or MMP-9 immunoreactivity in the respective enzyme-linked immunosorbent assays (ELISAs). The specimens were refrigerated after collection and centrifuged for 10 minutes at 2500 rpm at a temperature of 4° C. Following centrifugation, 0.25 ml of the supernatant was aliquotted into microfuge tubes and stored frozen at -80° C until assayed.

Commercially available ELISAs for MMP-1 and -9 (Oncogene, Cambridge, MA, USA) were used to measure the MMP-1 and-9 in the cervicovaginal secretion supernatants. The intra-assay coefficient of variability (CV) was 6%, and the interassay CV was 12% CV for both biochemical endpoints. The lower limit of detection of MMP-1 and -9 was 0.055 ng/ml with values below this designated as undetectable. For analysis, we used the value of 0 ng/ml for the undetectable samples. There was no significant difference between samples collected in the Netherlands and in New York. For statistical analysis the χ² test was used for evaluation of rates and proportions. Continuous variables were compared by the Wilcoxon signed-rank sum tests, with two-tailed α < 0.05 considered significant.

Results

Table 1 illustrates the demographic and obstetric characteristics of the 32 women who delivered preterm and in the study population 80 age, race and gestational age matched controls that delivered at term. In the study population the total cervicovaginal samples available for the assays were MMP-1 (n=190) and MMP-9 (n=107). Only 26% of all specimens had detectable levels of MMP-1. Among cases and controls, cervicovaginal MMP-1 levels were unaffected by labor. Table 2 demonstrates that, among non-laboring control (term) patients (> 3 weeks prior to delivery), MMP-9 concentrations were low. However, MMP-9 levels increased significantly within 1 week of term labor and during term labor. The highest MMP-9 values were observed among laboring patients with ruptured membranes.

Table 3 demonstrates that, among cases, MMP-9 levels were unaltered prior to preterm labor, but increased among patients in preterm labor. Figure 1 shows that the median MMP-9 levels were barely detectable during pregnancy, were
increased in preterm and term labor and displayed a more than ten-fold increase in the presence of ruptured membranes. The significant correlation between MMP-9 levels and cervical dilatation is shown in Figure 2 ($r = 0.48; p < 0.02$).

### Table 1. Sociodemographic characteristics of the study population for matrix metalloproteinase (MMP)-1 and MMP-9.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Preterm (n=32)</th>
<th>Term (n=80)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&gt;3 weeks prior to delivery</td>
<td>&lt;1 week prior to delivery</td>
</tr>
<tr>
<td></td>
<td>(n=19)</td>
<td>(n=7)</td>
</tr>
<tr>
<td><strong>Race</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Black</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Asian</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Hispanic</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>White</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td><strong>Parity</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nulliparous</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Multiparous</td>
<td>14</td>
<td>2</td>
</tr>
<tr>
<td><strong>Maternal age</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 20 yr.</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>20 &lt; 35 yr.</td>
<td>14</td>
<td>5</td>
</tr>
<tr>
<td>≥ 35 yr.</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td><strong>Number of patients assayed</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>for MMP-1</td>
<td>18</td>
<td>6</td>
</tr>
<tr>
<td><strong>Number of patients assayed</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>for MMP-9</td>
<td>3</td>
<td>6</td>
</tr>
</tbody>
</table>
Table 2. Median and range of matrix metalloproteinase (MMP)-9 values (ng/ml) in detectable cervicovaginal secretions collected more than 3 weeks and less than 1 week prior to delivery, and in term labor, with or without rupture of the membranes (ROM).

<table>
<thead>
<tr>
<th></th>
<th>&gt;3 weeks prior to delivery (n=28)</th>
<th>&lt;1 week prior to delivery (n=14)</th>
<th>In labor (n=28)</th>
<th>No ROM (n=20)</th>
<th>ROM (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMP-9</td>
<td>0 (0-0.04)</td>
<td>0.8 (0-22.8)</td>
<td>6.6 (0-30.6) *</td>
<td>1.3 (0-14.4)</td>
<td>26.5(15.5-30.6) †</td>
</tr>
</tbody>
</table>

*p<.001 by the Kruskal-Wallis multiple group rank sum test for the comparison of the three time period groups; † p<.001 by the Wilcoxon rank sum test for the comparison of the term ROM vs. no ROM groups.

Table 3. Median and range of matrix metalloproteinase (MMP)-9 values (ng/ml) in detectable vaginal secretions collected during pregnancy (non-labor) and in preterm labor.

<table>
<thead>
<tr>
<th></th>
<th>Preterm non-labor (n=9)</th>
<th>Preterm labor (n=5)</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMP-9</td>
<td>0 (0-0.14)</td>
<td>0.3 (0-30)</td>
<td>0.02</td>
</tr>
</tbody>
</table>

* By the Wilcoxon rank sum test
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Figure 1. Association of median matrix metalloproteinase (MMP)-9 during pregnancy, prior to delivery and in preterm and term labor with or without rupture of membranes.

Figure 2. Correlation between matrix metalloproteinase (MMP)-9 concentration and cervical dilatation in term labor with and without rupture of membranes (ROM) and in preterm labor without ROM.
Rajabi and colleagues found that MMP-1 levels were increased 13-fold in the human lower uterine segment and cervix at parturition. In human placentas a significant increase of total collagenase activity has been shown after the onset of labor. After inactivation of tissue inhibitor of MMP-1, a 12- to 17-fold increase in collagenase activity was found. Vadillo-Ortega and co-workers found increased levels of MMP-9 in amniotic fluid of patients with normal term labor and preterm rupture of membranes.

Elevated levels of collagenolytic activity, measured in cervical biopsies from pregnant women who delivered at term, indicate that collagenase may play an active role in the cervical ripening process. As collagenase acts extracellularly in the matrix, it might diffuse into the serum and provide a signal of incipient labor. Rajabi and associates showed no change in serum MMP-1 activity throughout pregnancy until the onset of term labor and an eight-fold increase during preterm labor. They suggested that serum MMP-1 might be a valuable marker for detecting preterm labor. Granström and colleagues reported that serum collagenase at term was higher than in the non-pregnant state. MMP-1 levels were higher in women with a ripe cervix than in those described as having stiff and inelastic cervices. Contrary to these reports, Morrison and co-workers found no elevation in serum collagenase at term labor. These inconsistent findings may be due to the use of different assay methods.

Immediately prior to and during labor, we expected increases in the cervicovaginal secretion of MMP-1. However, we now report that cervicovaginal MMP-1 was not associated with the onset of preterm or term labor. It is possible that MMP-1 activity is restricted to the tissue and not released into cervicovaginal secretions. Prior to accepting this conclusion, however, our cross-sectional sampling methodology should be compared with a longitudinal study, in which a group of women have their MMP levels measured over time, through labor and delivery. An additional caveat relates to the nature of the ELISA assay. Whereas total immunological levels of MMP generally correlate well with biological activity, it is possible that tissue inhibitors of metalloproteinase activity could have influenced our results. For example, MMP activity might be increased in the absence of elevated concentrations if inhibitor levels are reduced. Given our findings, the ELISA assay of vaginal MMP-1 does not seem useful for prediction of preterm delivery.
In our study, among controls, cervicovaginal MMP-9 values were low more than 3 weeks prior to the onset of term labor, increased within 1 week of labor, reached relatively high concentrations in labor and peaked with membrane rupture. In contrast, among cases, MMP-9 levels remained unchanged until the onset of spontaneous preterm labor.

The presence of MMP-9 has been studied in rat amnion, human amniochorion, amniotic fluid, urine, and plasma. Vadillo-Ortega and colleagues demonstrated with Western blot analyses that MMP-9 protein increased in the human amniochorion with labor. MMP-9 is expressed by amnion epithelium, macrophages, chorion laeve trophoblast and decidual cells. The increased expression of MMP-9 will result in degradation of the extracellular matrix of the fetal membranes and facilitate their rupture under both physiological and pathological conditions. Athayde and co-workers showed that women with preterm premature rupture of membranes had higher MMP-9 concentrations in amniotic fluid than women in spontaneous preterm labor with intact membranes. Women with an intra-amniotic infection had higher concentrations of MMP-9 than women without infection. Locksmith and associates found MMP-9 levels in the amniotic fluid to be reliable for diagnosing intra-amniotic infection. Recently, a simple non-invasive assay was used to quantitate urinary MMP-9 activity among a small group of patients (n=15) with threatened premature labor. This study showed that both positive and negative predictive values for a risk of premature delivery were 80%. Tu and co-workers reported that plasma MMP-9 concentrations, assayed with an ELISA, remained unchanged throughout pregnancy, but increased in women who presented with spontaneous labor.

The timing of MMP-9 expression in the cervicovaginal secretions in our study is comparable with the findings of Tu and colleagues in plasma. We also showed an association of cervicovaginal MMP-9 expression with rupture of the membranes, as was previously described in plasma and amniotic fluid. We anticipated that preterm labor would be preceded by a prolonged period of cervical remodelling and ripening, causing earlier release of MMP-9 into cervicovaginal secretions than was found preceding term labor. However, our results indicate that term labor was preceded by earlier release of MMP-9. We did find a significant correlation between MMP-9 expression and cervical dilatation.
While, there might be an important role for MMP-9 in the mechanisms of labor and membrane rupture, MMP-9 levels prior to preterm delivery are not significantly increased in comparison to MMP-9 throughout pregnancy. MMP-9 therefore does not appear to be useful as a predictor of preterm birth. However, since we measured MMP-9 in the same manner as MMP-1, i.e. with an ELISA, the limitation discussed above applies to MMP-9 as well. Interestingly, MMP-9 might be useful to confirm a suspicion of premature rupture of membranes if amniotic fluid cannot be collected for a confirmatory fern or pH test. Future research is necessary to study the usefulness of such a confirmatory test.
References

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