Preterm delivery is one of the main causes of perinatal mortality and morbidity worldwide. It is an escalating problem in Czech Republic and has increased to 6%. Premature rupture of the membranes is defined as fetal membranes rupture with leakage of amniotic fluid that precedes the onset of uterine contraction by at least 2 hours. It complicates 4–7% of all births and is directly associated with a short length of gestation and an increased perinatal morbidity and mortality. There are predisposing conditions connected with the occurrence of premature rupture of the membranes such as local infections, cervical incompetence and low socio-economic condition (5, 13). Even so, the aetiology still remains unknown in the majority of cases. Increased biosynthesis of prostaglandins by intrauterine tissue is widely accepted as a key event in the initiation of parturition (8). Preterm labour after spontaneous rupture of the membranes is associated with a significant increase in amniotic fluid concentration of prostaglandins. A main reason of this elevation is probably infection. The purpose of this study was to evaluate the prevalence of cervical colonization by genital mycoplasmas in patients with preterm premature rupture of the membranes (PPROM). Cervical swabs were obtained for genital mycoplasmas and standard vaginal smears of bacterial culture were performed at the time of patients' admission. In the control group were 225 women with a normal pregnancy. Results: Ureaplasma urealyticum was detected in 68% (152/225) and Mycoplasma hominis was detected in 28% (63/225) of the patients with PPROM between 24 and 36 weeks of gestation and. In the control group Ureaplasma urealyticum was found in 17% (38/225) and Mycoplasma hominis in 15% (35/225) pregnant women. Conclusion: Our results provide evidence of an association between cervical colonization with genital mycoplasmas and preterm premature rupture of the membranes.

Key words: Cervical colonization; Genital mycoplasmas; Preterm premature rupture of the membranes
verse neonatal outcomes. Clinical management in the study group was performed according to standard protocols at our department. The antibiotics were given after collecting the cervical swab and the vaginal smear.

**Statistical methods**

Statistical analyses were performed using Fischer’s exact tests. A probability value of <0.05 was considered significant.

**Culture procedures**

The Mycoplasma IST 2 kit (Biomérieux, France) was used to diagnose the mycoplasmal infections. It enables culture, identification, indicative enumeration and antibiotic susceptibility testing (with 9 antibiotics – doxycycline, josamycin, ofloxacin, erythromycin, tetracycline, ciprofloxacin, azitromycin, clarytromycin and pristinamycin) of Ureaplasma urealyticum (Uu.) and Mycoplasma hominis (Mh.). It combines a selective culture broth with a strip containing 22 tests. This kit allows pathogen identification within 48 hours and determines the amount of bacteria, thus making differentiation possible between colonization and infection (cell count above 10^4 is the evidence of infection). The combination of three antibiotics and one antifungal agent provides selectivity, ensuring that any contaminating flora present in the specimen does not affect the test. After the collecting the sample the cervical swab for Uu. and Mh. was inserted into the Biomérieux transport medium R1, provided with Mycoplasma IST 2 kit. The swab was stored in the transport medium at 4 °C until transported to the laboratory. All the swabs were processed in the laboratory within 5 hours of collection. The results were interpreted after 24 hours and 48 hours of incubation.

**Diagnosis of amnionitis chorioamnionitis, and funisitis**

Histologic chorioamnionitis was defined as the presence of acute inflammatory changes on examination of a membrane roll and chorionic plate of the placenta; funisitis was diagnosed in the presence of neutrophil infiltration into the umbilical vessel walls or Wharton’s jelly. Amnionitis was defined as the presence of acute inflammatory changes in the membranes.

**Results**

**Colonization of the cervix with genital mycoplasmas**

In the normal pregnancy group, 32 % (73/225) of the women in this group had a positive cervical culture for genital mycoplasmas. In contrast, the cervical colonisation ratio for genital mycoplasmas in women with PPROM was 96 % (215/225) – Tab. 1, demonstrating a significant difference between groups with PPROM and control group (p < 0.0001, by Fisher’s exact test).

**Maternal serum C - reactive protein in cases with PPROM**

Maternal serum C - reactive protein (CRP) levels were divided into 3 group: negative (0–5 mg/l), mild positive (6–19 mg/l) and positive (20 and more mg/l). A negative CRP was encountered in 56 % (126/225), mild positive in 10 % (22/225) and a positive result was found in 34 % (77/225).

**Comparison of maternal serum C-reactive protein in cases of PPROM and histopathologic findings on the placenta and the membranes**

We compared maternal serum CRP in cases of PPROM and histopathological findings on the placenta and the membranes.

<table>
<thead>
<tr>
<th>Without inflammatory changes</th>
<th>Amnionitis</th>
<th>Chorioamnionitis</th>
<th>Funisitis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative CRP</td>
<td>56 (44 %)</td>
<td>22 (18 %)</td>
<td>19 (15 %)</td>
</tr>
<tr>
<td>Mild positive CRP</td>
<td>7 (32 %)</td>
<td>0 (0 %)</td>
<td>11 (50 %)</td>
</tr>
<tr>
<td>Positive CRP</td>
<td>24 (31 %)</td>
<td>1 (1 %)</td>
<td>24 (31 %)</td>
</tr>
</tbody>
</table>

**Tab. 3:** The histopathologic findings on the placenta and the membranes in cases of PPROM with positive genital mycoplasmas (p <0.0047).

<table>
<thead>
<tr>
<th>Without inflammatory changes</th>
<th>Amnionitis</th>
<th>Chorioamnionitis</th>
<th>Funisitis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive genital mycoplasmas</td>
<td>81 (36 %)</td>
<td>19 (8 %)</td>
<td>54 (24 %)</td>
</tr>
<tr>
<td>Negative genital mycoplasmas</td>
<td>6 (3 %)</td>
<td>4 (2 %)</td>
<td>0 (0 %)</td>
</tr>
</tbody>
</table>
membranes. Patients with a negative serum CRP did not have inflammatory changes on the placenta and the membranes in 44 % (56/126); amnionitis was found in 18 % (22/126) chorioamnionitis was found in 15 % (19/126), funisitis in 23 % (29/126). In patients with a positive serum CRP; 31 % (24/77) did not have any inflammatory changes; amnionitis was in 1 % (1/77) chorioamnionitis was in 31 % (24/77) and funisitis in 37 % (28/77) (Tab. 2). A statistical difference was found (p < 0.0138, by Fischer’s exact test).

Histopathologic findings on the placenta and the membranes in cases of PPROM with positive genital mycoplasmas

The prevalence of chorioamnionitis and funisitis was 25 % (54/215) and 28 % (61/215) respectively in cases of PPROM with positive genital mycoplasmas (Tab. 3). A statistical difference was found (p< 0.0047, by Fischer’s exact test).

Discussion

Genital mycoplasmas compose the microorganisms that are the most frequently isolated from both placental membranes and amniotic fluid in women with histological and clinical chorioamnionitis (5). These organisms are commonly found within the uterus in association with spontaneous preterm labor and with PPROM (13). The intrauterine presence of these organisms has been associated with an increased production of a wide variety of cytokines, matrix metalloproteinases, and prostaglandins, all believed to be in the causal pathway and/or precursors for spontaneous labor and PPROM (8). Ureaplasma urealyticum (Uu.) has been detected in the lower genital tract of 40 % to 80 % sexually active women depending on geographical areas, and appears to be common in the vaginal flora of pregnant women (6). Witt et al described microbial invasion of the amniotic cavity with Uu. in 43.9 % patients with preterm labor (14). Vice versa genital mycoplasmas are the organisms most frequently isolated from the amniotic fluid in patients with preterm and term gestations, and they have been implicated in the pathogenesis of clinical chorioamnionitis, puerperal endometritis, postoperative wounds infections, neonatal sepsis, meningitis and in the genesis of chorioamnionitis, puerperal endometritis, postoperative wounds inflammatory changes in the placenta and membranes. We studied 225 women between 24 and 36 weeks of gestation with PPROM. In the control group the prevalence of colonization of the cervix with genital mycoplasmas during pregnancy was 32 %. In contrast, women with PPROM were found to have a cervical colonization rate with genital mycoplasmas of 96 %. Our results provide strong evidence of an association between cervical colonization with genital mycoplasmas and PPROM. Maternal serum CRP level did not correlate with histologically confirmed inflammatory changes in the placenta and membranes. We recommended early antibiotic treatment against the genital mycoplasmas in all the cases of PPROM.

Conclusion

We studied 225 women between 24 and 36 weeks of gestation with PPROM. In the control group the prevalence of colonization of the cervix with genital mycoplasmas of 96 %. Our results provide strong evidence of an association between cervical colonization with genital mycoplasmas and PPROM. Maternal serum CRP level did not correlate with histologically confirmed inflammatory changes in the placenta and membranes. We recommended early antibiotic treatment against the genital mycoplasmas in all the cases of PPROM.

References


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