Chlamydia and mycoplasma infections during pregnancy and their relationships to orofacial cleft

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Abstract. Serum antibodies to Mycoplasma pneumoniae and Chlamydia trachomatis have been studied in a group of newborns with orofacial cleft (OC) and their mothers (n = 59) as compared to a control group of healthy newborns and their mothers (n = 40) assayed by ELISA and Western blot analysis. In the first group, IgG antibodies to M. pneumoniae were found by ELISA in 12 newborns with OC and 22 mothers, while IgA antibodies were detected only in 5 and 11 cases, respectively. IgM antibodies indicating an acute infection were found in 2 mothers only. IgG antibodies to C. trachomatis were found in 2 newborns with OC and 4 mothers. In the control group, IgG antibodies to M. pneumoniae were found in 3 newborns and 7 mothers. IgG antibodies to C trachomatis were observed in 1 newborn and 1 mother, while IgM antibodies to C trachomatis were present in 1 mother only. Immunoblot analysis revealed in newborns with OC and their mothers C. trachomatis-specific bands associated with MOMP 1, 29 kD, 45 kD, and heat shock proteins (HSP) 60 and 70. Based on these results we suggest that the risk associated with exposure to M. pneumoniae and/or C. trachomatis is so far unknown and further study is needed for its estimation.

Key words: antibodies C. trachomatis, M. pneumoniae, mothers, newborns, orofacial cleft.

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Introduction

The pathogenesis of congenital defects developing after an intrauterine infection with viruses and bacteria are still in the scope of interest of many specialists. The effects of a chlamydial infection on the newborn are well characterized. Usually, if the mother is infected, her newborn acquires the infection during the delivery. Conjunctivitis develops in up to 50% of infected newborns and pneumonia in 10-20%, but little is known about the effect of such an infection on development of congenital defects.

Recently, several investigators have reported a higher incidence of intrauterine infections with chlamydia and mycoplasma (FEJGİN et al., 1997; DONG et al., 1998; GONCALVES et al., 2002), which might result in congenital defects (DIMITRAKOV et al., 2000; MOLNÁROVÁ et al., 2005). Chlamydiae pass through fetal membranes (FRAIZ and JONES, 1988; DONDERS et al., 1991) and can be isolated from amniotic fluid (PAO et al. 1991; DJUKIC et al., 1996). Intrauterine infections with chlamydia and mycoplasma result in low birth-weight infants (DONDERS et al., 1991; RASTOGY et al., 1999), abortions (WITKIN et al., 1992; REN et al., 1997; KISHORE et al., 2003), preterm delivery and stillbirth (GENCAY et al., 2001), and perinatal death (GENCAY et al., 1997, 2000; RASTOGY et al., 1999).

Even though chlamydia are obligate intracellular bacteria, their clinical features, pathogenesis, pathology and epidemiology are similar to those of viral infections. Mycoplasmas are essentially bacteria lacking a rigid cell wall during their entire life cycle, although they are much smaller than bacteria. Infections caused by these microorganisms have many common features, namely similar clinical symptoms in the urogenital tract (pelvic inflammatory disease, vaginitis, cervicitis, urethritis and pyelonephritis) and the respiratory tract as well (KAZÁR et al., 1992; MEDKOVÁ, 2004). Rhinitis, bronchitis and infantile pneumonia (HOLČÍKOVÁ and MEDKOVÁ, 2000), and neonatal inclusion conjunctivitis have been found too (SANDSTROM et al., 1984; MARD, 2002).
In this study, we tried to assess whether specific antibodies are formed in sera of mothers and their newborns with OC in response to *M. pneumoniae* and *C. trachomatis* infections as compared to healthy newborns and their mothers.

**Materials and Methods**

*Serum samples* from newborns with OC (Fig.2) and their mothers (59 each) with typical signs of infection caused by chlamydia and/or mycoplasma (pelvic inflammatory disease) and from healthy newborns and their mothers (40 each) were collected at the Plastic Surgery Department of the Ruzinov Hospital, Bratislava, Slovak Republic, in 2003-2004. In the group of newborns with OC and their mothers, *C. trachomatis* was detected only in mothers, but their newborns developed conjunctivitis, bronchitis or pneumonia.

**ELISA** a commercial kit (*Mycoplasma pneumoniae* – IgG and IgA-ELISA, Medac, Germany) was used to assay IgG, IgA and IgM antibodies to *M. pneumoniae*. For detection of IgG, IgA and IgM antibodies to *C. trachomatis*, another commercial kit (*Chlamydia trachomatis* – IgG and IgA pELISA, Medac, Germany) was employed. Cut-off values for both assays were calculated according to the manufacturer’s instructions.

**Immunoblot analysis** was performed by means of a commercial kit (AID GmbH, Germany). It detected IgG and IgA antibodies to *C. trachomatis*.

**Statistical analysis** was performed by the chi-square test. A difference with P value ≤ 0.05 was considered significant.
Results

ELISA

In testing antibodies to *M. pneumoniae* (n = 59), 12 (20.0%) newborns with OC and 22 (37.3%) mothers were positive for IgG antibodies, 5 (8.5%) newborns with OC and 11 (18.6%) mothers were positive for IgA antibodies, and no newborn with OC and 2 (3.4%) mothers were positive for IgM antibodies (Table 1). Only the difference between the IgG positivity of newborns with OC and that of their mothers was significant. In the control group (n = 40), 3 (7.5%) healthy newborns and 7 (17.5%) mothers had IgG antibodies, while IgA and IgM antibodies were not detected (Table 2.).

Regarding the antibodies to *C. trachomatis*, 2 (3.4%) newborns with OC and 4 (6.8%) mothers were positive for IgG antibodies, 1 (1.7%) newborn with OC and 1 (1.7%) mother were positive for IgA antibodies, but none had IgM antibodies (Table 1). In the control group, 1 (2.5%) healthy newborn and 1 (2.5%) mother were positive for IgG antibodies, 1 (2.5%) mother had IgM antibodies, but none had IgA antibodies (Table 2).

Immunoblot analysis

The IgG and IgA immune responses to *C. trachomatis* in newborns with OC and their mothers were further examined by immunoblot analysis (Fig. 1). The latter showed specific bands associated with 40 kD (MOMP 1), 38 kD (MOMP 1’), and a 29 kD protein in both newborns with OC and their mothers. HSP 60 and HSP 70, which are not regarded as species-specific but important for the recognition of chronic stage of infection, were seen in 75% serum samples. The presence of MOMP 1, detected with both IgG and IgA antibodies in one serum pair (lane 4), indicated an acute infection. An infection, which had been contracted by the individual apparently long time ago and is characteristic by the presence of MOMP 1, HSP 60, and HSP 70, all detectable by the IgG and IgA antibodies were seen in three and one mothers sample, respectively (lanes 1,3,5).
Discussion

In this study, the prevalence of antibodies to *M. pneumoniae* among newborns with OC and their mothers was rather high. Out of 59 samples of mothers and their newborns with OC, 37.3% of mothers had serological evidence of infection with *M. pneumonia*, whereas only 20% of infants had specific antibodies. Simultaneously IgG and IgA antibodies were confirmed in 18.6% and 8.5% of mothers and newborns serum samples. IgM antibodies, indicating acute infection, were detected in 3.4% of mothers but not in their newborns. In contrast, *C. trachomatis* IgG antibodies were found in 3.4% and 6.8% newborns with OC and their mothers, compared with 2.5% of healthy newborns and mothers. Only one specimen of mothers contained IgM antibodies.

The immunoblot analysis revealed the 40 kD MOMP 1 of *C. trachomatis* as principal immunoreactive component observed in both IgG and IgA antibody responses. Further most frequent group of antibodies recognized HSP 60, which is described as immunopathogenic in chlamydial infection. (BIENDO et al., 1996).

The results of this study suggest a more frequent occurrence of a *M. pneumoniae* infection compared with a *C. trachomatis* one in the group of newborns with OC and their mothers. The low prevalence of *C. trachomatis* infection suggests that the bacterium is probably not an important risk factor in the OC occurrence.

Chlamydial or mycoplasmal infections during pregnancy may have devastating effects on pregnancy outcome and represent a major public health problem worldwide. Stillbirth, preterm delivery or perinatal death (GENCAY et al., 1997; GENCAY et al., 2000; FEJGIN et al., 1997; DONG et al., 1988; RASTOGI et al., 1999; WITKIN et al., 1992; REN et al., 1997) as well as neonatal conjunctivitis or pneumonia (MARDH, 2002; SANDSTROM et al., 1984) has been reported. (THORP et al., 1989) have found that chlamydia pass through fetal membranes and thus cause pneumonia. An intrauterine infection with *C. trachomatis* may result in an infection of respiratory tract at early neonatal stage (NUMAZAKI et al. 2003). *C. trachomatis* has been
observed, besides in amniotic fluid and chorionic villi (PAO et al., 1991; DJUKIC et al., 1996), also
in placenta (GENCAY et al., 1997) and cervix (Vile et al., 1997). Endometritis after childbirth was
much more frequent if associated with *C. trachomatis* infection of mothers (WITKIN et al., 1992;
PAUKKU et al., 1999).

Birth of infants with congenital defects caused by intrauterine chlamydial infection is not
excluded. *C. trachomatis*-specific DNA has been detected in the urine and the cerebrospinal fluid
from neonates with hydrocephalus and in the urine of their mothers. Electron microscopic
examination showed typical chlamydial inclusions and the titer of specific IgM antibodies in
neonate reached 600 (DIMITRAKOV et al., 2000).

Since the incidence of antibodies to *C. trachomatis* is low, this microorganism apparently
does not influence the development of OC. These results are confirmed with those of other studies
(RAE et al., 1994; OSJER et al., 1996; SOZIO et al., 1998), in which no devastating effect of the
infection with *C. trachomatis* during pregnancy was observed.

As to the infection with *M. pneumoniae* its possible harmful effect on newborns is
ambiguous. LABBE et al. (2002) and KOVACHEV et al. (2002) did not find any adverse effect of
the infection on pregnancy, while DONDE RS et al. (2000), WASIELA et al. (2003), PERNI et al.
(2004), ODENDAL et al. (2002), and NGUYEN et al. (2004) have noted a preterm spontaneous
delivery. Besides, ANGOULVANT et al. (2000) have found a relationship between the Gianotty
Crosti syndrome and the infection with *M. pneumoniae*. The data obtained in this study is not
conclusive enough to establish that the infection of mothers with mycoplasma and/or chlamydia is
transmitted to the fetus and results in the development of OC, and further studies are needed to
elucidate this issue.

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References


Table 1. Serum IgG, IgA and IgM antibodies to *M. pneumoniae* and *C. trachomatis* in newborns with OC and their mothers as assayed by ELISA

<table>
<thead>
<tr>
<th>Globulin class</th>
<th>Antibodies to <em>M. Pneumoniae</em></th>
<th>Antibodies to <em>C. trachomatis</em></th>
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<td>Positive newborns [No (%)]</td>
<td>Positive mothers [No (%)]</td>
</tr>
<tr>
<td>IgG</td>
<td>12 (20.0)</td>
<td>22 (37.3)</td>
</tr>
<tr>
<td>IgA</td>
<td>5 (8.5)</td>
<td>11 (18.6)</td>
</tr>
<tr>
<td>IgM</td>
<td>0</td>
<td>2 (3.4)</td>
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</tbody>
</table>

NS = non-significant
Table 2. Serum IgG, IgA and IgM antibodies to *M. pneumoniae* and *C. trachomatis* in healthy newborns and their mothers as assayed by ELISA

<table>
<thead>
<tr>
<th>Globulin class</th>
<th>Antibodies to <em>M. Pneumoniae</em></th>
<th>Antibodies to <em>C. trachomatis</em></th>
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NS = non-significant
Fig. 1 Immunoblot analysis of sera from newborns with OC and their mothers

Four paired sera (positive for *C. trachomatis* by ELISA) were examined. IgG and IgA antibodies were directed to the proteins of 40 kD (MOMP 1), 38 kD (MOMP 1'), 29 kD, 60 kD (HSP 60) and 70 kD (HSP 70). Size markers are on the left.
Fig. 2. Newborn with orofacial cleft – cleft lip and palate (A); B (detail).