Chlamydia trachomatis infection in pregnant women with premature membrane rupture or premature delivery threat.


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SUMMARY.
Introduction. *Chlamydia trachomatis* is considered the causal agent of trachoma, salpingitis, endometritis, and may be involved in premature membrane rupture (PMR) and premature delivery threat (PDT). The aim of this work was to determine the presence of *C. trachomatis* antigens and antibodies against *C. trachomatis* in pregnant women with PMR, PDT or normal pregnancy (NP).

Material and methods. We took endocervical samples from 50 pregnant women of each group for determination of *C. trachomatis* antigens by means of an direct immunofluorescence method; additionally, 5 mL of peripheral blood were taken to identify anti-*C. trachomatis* antibodies by indirect immunofluorescence assay.

Results. Six per cent (3/50) of PMR patients showed *C. trachomatis* antigens and IgG anti-*C. trachomatis* antibodies. Two per cent (1/50) of PDT patients had *C. trachomatis* and IgM anti-*C. trachomatis* antibodies. Six per cent (3/50) of NP patients exhibited antigens *C. trachomatis* but no anti-*C. trachomatis* antibodies. Moreover, only IgG anti-*C. trachomatis* antibodies were found respectively in 10% (5/50), 10% (5/50) and 16% (8/50) of the PMR, PDT and NP women groups.

Conclusions. The finding of *C. trachomatis* antigens as well as anti-*C. trachomatis* antibodies in the three studied groups, emphasizes the importance of an opportune identification of the infection in order to apply the adequate treatment and prevent sequelae in both the pregnant women and their products.

Key words: *Chlamydia trachomatis*, infection, pregnancy, premature membrane rupture, premature delivery threat.
RESUMEN.
Chlamydia trachomatis en mujeres embarazadas con ruptura prematura de membranas o amenaza de parto prematuro.
Introducción. Chlamydia trachomatis se considera el agente causal de tracoma, salpingitis, endometritis y podría estar involucrada en la ruptura prematura de membrana (PMR) y amenaza de parto prematuro (PDT). El objetivo de este trabajo fue determinar la presencia de antígenos de C. trachomatis y anticuerpos contra C. trachomatis en mujeres embarazadas con PMR, PDT (ambos grupos de etiología desconocida) y mujeres con embarazo normal (NP).
Material y métodos. Se obtuvieron 50 muestras endocervicales por cada grupo de mujeres embarazadas, para la determinación de antígenos de C. trachomatis, por el método de inmunofluorescencia directa. Asimismo fueron tomados 5 ml de sangre venosa, para identificar la presencia de anticuerpos contra C. trachomatis por inmunofluorescencia indirecta.
Resultados. Seis por ciento (3/50) de las pacientes con PMR presentaron antígenos de C. trachomatis y anticuerpos IgG anti-C. trachomatis. Dos por ciento (1/50) con PDT tuvieron antígenos de C. trachomatis y anticuerpos IgM anti-C. trachomatis. Seis por ciento (3/50) de las pacientes con NP mostraron antígenos de C. trachomatis, pero no anticuerpos anti-C. trachomatis. Sin embargo, en 10% (5/50), 10% (5/50) y 16% (8/50) con PMR, PDT o NP, respectivamente; solamente se encontraron anticuerpos IgG anti-C. trachomatis.
Conclusión. El hallazgo tanto de antígenos como anticuerpos anti-C. trachomatis en los tres grupos estudiados, resalta la importancia de la oportuna identificación de la infección, para la aplicación del tratamiento adecuado, para prevenir las secuelas de la infección, tanto en las mujeres embarazadas como en sus productos. (Rev Biomed 2001; 12:158-165)

Palabras clave: Chlamydia trachomatis, infección, embarazo, ruptura prematura de membranas, amenaza de parto prematuro.

INTRODUCTION.
Chlamydia trachomatis, an obligate intracellular bacterium, is one of the most common agents of sexually transmitted diseases in both industrialized and developing countries (1-4). In Mexico, there are no reliable official statistics, although diverse studies have been carried out to assess the extent of the problem (5-10). The prevalence of C. trachomatis infection in the Mexican population is variable depending on the level of attention and the clinical status of the patients (6,8,9).

The etiology of gestational and prenatal disturbances is diverse (8,11-14). The majority of these disturbances are related to bacterial infections of the urogenital tract (5,15-18). C. trachomatis infection has been associated with premature delivery threat, ectopic pregnancy and recurrent abortion (2,7,19-24). Non treatment of C. trachomatis infection in pregnant women can provoke conjunctivitis or pneumonia in the product at birth (19,21,25). The sequelae of C. trachomatis infection in the women include pelvic inflammatory disease (20,26,27) and infertility (23,28). The diagnosis of C. trachomatis infection is difficult since 70%-90% of the endocervical chlamydial infections in women are asymptomatic and may persist for months to years (1,29,30). The main identification procedures of C. trachomatis are cultures in HeLa or McCoy's cells (24,31), fluorescent antibodies assay (32), enzyme immunoassays (EIA) (33), ligase chain reaction (LCR), polymerase chain reaction (PCR) (32,34-36) and genomic DNA analysis (37). This work had the objective of investigating to detect the presence of C. trachomatis antigens or antibodies against C. trachomatis in pregnant women with PMR or PDT by direct and indirect immunofluorescence methods respectively.
PATIENTS AND METHODS.

One hundred and fifty pregnant women receiving medical attention at the Gineco-Obstetric Hospital of the Medical National Center of Occident, IMSS, Mexico, were studied. Fifty women with PMR and fifty with PDT were chosen from the High Risk Pregnancy Service, without knowing the cause of their pathology. Fifty women with NP were from the Outpatient Service of the same Hospital. A clinical record was elaborated for each patient, which included age, number of pregnancies, sexual partners, weeks of gestation, abortion, perinatal infections. They were informed about the aims of the necessity of obtaining biological samples from them. Their approval was solicited and obtained.

Sample procedure. In accordance with the suppliers' instructions, we proceeded to obtain an endocervical sample. The cervix was cleaned with sterilized gauze. A large swab was introduced one centimeter into the endocervical channel and rotated 5-10 seconds to gently detach epithelial cells. The swab was withdrawn without touching the vaginal walls. All hemorrhagic and pustulous samples were discarded. Immediately after the sampling, two glass-slide frotis were set. The swab was rotated inside the circular mark on the glass-slide (bioMérieux 55331). All samples were fixed with acetone, and stored at –20°C until further processing. Besides, 5 ml of peripheral venous blood was also taken and the obtained serum was kept frozen.

Identification of C. trachomatis by direct immunofluorescence (DIF). In agreement with the equipment supplier we proceeded to determine C. trachomatis. All glass-slide endocervical samples were covered with 20 µl of monoclonal murine anti-C. trachomatis antibodies (bioMérieux 55331) and incubated at room temperature in a humid chamber for 15 min. Thereafter the glass-slide were washed twice with phosphate buffer saline (PBS) solution, drained and covered with glycerol-PBS and a coverslide. They were placed in darkness for an hour, and then observed with an epifluorescence microscope (40X). Samples exhibiting ten or more fluorescent chlamydial bodies (elemental or reticulate bodies) per field were declared positive. As a reference we used mammalian cells (negative control) or mammalian cells with chlamydial bodies (positive control) (bioMérieux 55321). The results were expressed qualitatively as number and percent of positive cases per group.

Determination of anti-C. trachomatis antibodies. The procedure was carried out in accordance with the equipment supplier's instructions. Anti-C. trachomatis antibodies were first evidenced by indirect immunofluorescence (IIF) as total immunoglobulins (Igs) (bioMérieux 75603). Positive samples with anti-C. trachomatis antibodies at > 1:124 dilution were processed to determine the class of antibody present in the samples (IgG bioMérieux 75692 or IgM bioMérieux 75692). The general procedure was the following: all pregnant women serum samples were diluted with PBS 1:124. Twenty µl of each sample were deposited on a glass-slide (bioMérieux 72051) containing Chlamydia trachomatis-Spot IF serotype L2 (prebound and inactivated antigen) and were incubated at 37°C in a humid chamber. The glass-slides were rinsed twice with PBS. Later, conjugated murine antibody against human immunoglobulin (Igs, IgG or IgM) was added to the first antibody. The glass-slides were reincubated, rinsed twice and mounted with a fixing solution. They were observed with an epifluorescence microscope with an objective 40x. All samples displaying the “stared sky” aspect, with fluorescent green points over a red background at ≥ 1:124 dilution, were considered positive. The results were reported as number and percentage of positive cases per group.

Statistical analysis. Student’s t test was used to compare means, with a ”p” value <0.05 for
statistical significance.

**RESULTS.**

The background characteristics of the patients with PMR, PDT and NP are shown in table 1. Age, number of pregnancies, sexual partners and weeks of gestation, did not differ significantly among the groups. There was no association of these variables with the results of the determination of antigens of *C. trachomatis* or antibodies anti-*C. trachomatis* probably because of the small number of positive cases in the groups with PMR and PDT (table 3).

In table 2 it can be appreciated that out of a total 150 cases, only (7/150) 4.6% were positive to antigens of *C. trachomatis* whereas in the determination of antibodies anti-*C. trachomatis* there were (19/150) 12.6% positives cases. It is important to mention that the determination of antibodies anti-*C. trachomatis* revealed a higher prevalence of positives cases to *C. trachomatis*. Furthermore, determination of antibodies allowed to define the class of antibodies present in the positive cases (table 3), which in turn allowed to determine the evolution of the infection.

Table 3 shows that (3/50) 6% of the patients from the PMR group presented both *C. trachomatis* antigens and anti-*C. trachomatis* IgG+ antibodies. These indicate the presence of an active infection. The single patient in the PDT group who presented *C. trachomatis* antigens and IgM+ antibodies; probably had a recent infection. Six percent (3/50) in the NP group only presented *C. trachomatis* antigens, a finding suggestive of an incipient infection. Finally 4% (2/50), 10% (5/50) and 16% (8/50) of women from groups PMR, PDT and NP respectively had only IgG+ anti-*C. trachomatis* antibodies. This could mean the presence of a chronic infection or an immunologic scar from previous contact with *C. trachomatis*.

**DISCUSSION.**

The study of *C. trachomatis* infections in women can be focused in different ways. On one hand, the risk factors propitiating the onset of the infection such as age, beginning of sexual life, socioeconomic level and sexual partners can be studied (9,38). On the other hand, sequelae of chlamydial infections in women are considered risk factors for the development of other gynaecologic pathologies, such as pelvic inflammatory disease, ectopic pregnancy, and infertility (8,20,26-28).

### Table 1

**Main clinical characteristics of the pregnant women.**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>PMR (n=50)</th>
<th>PDT (n=50)</th>
<th>NP (n=50)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)*</td>
<td>26.9±5.8</td>
<td>25.7±5.4</td>
<td>24.8±5.7</td>
<td>NS</td>
</tr>
<tr>
<td>Pregancies*</td>
<td>2.9±2.1</td>
<td>2.4±1.4</td>
<td>2.4±1.7</td>
<td>NS</td>
</tr>
<tr>
<td>Abortion %</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Perinatal infection%</td>
<td>1.02±0.1</td>
<td>1.04±0.01</td>
<td>1.06±0.2</td>
<td>NS</td>
</tr>
<tr>
<td>Gestation (weeks)*</td>
<td>31.9±2.3</td>
<td>33.3±3.4</td>
<td>34.4±2.3</td>
<td>NS</td>
</tr>
</tbody>
</table>

* mean ± S D. p = t “student”.

**Table 2**

**Determination of *C. trachomatis* antigens or antibodies in pregnant women.**

<table>
<thead>
<tr>
<th>Test</th>
<th>PMR (n=50)</th>
<th>PDT (n=50)</th>
<th>NP (n=50)</th>
<th>Total (n=50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antigen + DIF</td>
<td>3</td>
<td>1</td>
<td>3</td>
<td>7 (4.6%)</td>
</tr>
<tr>
<td>Antibodies + IIF</td>
<td>5</td>
<td>6</td>
<td>8</td>
<td>19 (12.6%)</td>
</tr>
</tbody>
</table>

**Table 3**

**Presence of *C. trachomatis* antigens and/or anti-*C. trachomatis* antibodies in pregnant women.**

<table>
<thead>
<tr>
<th>Infection</th>
<th>Antigens</th>
<th>PMR (n=50)</th>
<th>PDT (n=50)</th>
<th>NP (n=50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlamydial Active</td>
<td>Ag + IgG+</td>
<td>3 (6%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Chronic/past</td>
<td>Ag – IgG+</td>
<td>2 (4%)</td>
<td>5 (10%)</td>
<td>8 (16%)</td>
</tr>
<tr>
<td>Reactive /</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>incipient</td>
<td>Ag + IgG-</td>
<td>0</td>
<td>0</td>
<td>3 (6%)</td>
</tr>
<tr>
<td>Recent</td>
<td>Ag + IgM+</td>
<td>0</td>
<td>1 (2%)</td>
<td>0</td>
</tr>
</tbody>
</table>

Ag = antigen.
Opportune detection of *C. trachomatis* infection during pregnancy will allow effective treatment in order to avoid complications such as abortion (23), a low-product-weight (8,10,14), and a premature delivery threat (12,18) as well as transmission of the infection to the newborn during the passage through the infected cervix (13,17,39).

At present, a controversy persists about the most effective method to identify *C. trachomatis* (1,6,39-44) given the differences in the sensibility and specificity of molecular methods, fluorescent antibody assays and enzyme immunoassays(40-52). However, the cellular culture for *C. trachomatis* is a highly specific method and is considered as the “gold standard” test (1). It is opportune to mention that the course of *C. trachomatis* infection can be monitored through the determination of the antigen or the bacterium itself, as well as through anti-*C. trachomatis* antibodies.

The purpose of this work was to investigate the correlation between *C. trachomatis* infection and PDT or PMR in patients without knowing the cause of their pathology. The results showed a weak association between the infection and PMR or PDT groups. Six per cent and 2% of the woman included in these groups presented *C. trachomatis* antigens and anti-*C trachomatis* antibodies (IgG or IgM). Moreover, 4% of the group of PMR and 10% of the group PDT only presented anti-*C. trachomatis* antibodies (IgG). A connection between *C. trachomatis* infection and PMR and PDT has been suggested but up to now there is no formal data to support this association(13,14,17,18,23,26).

It has been observed that the other cervical pathologies, increase the risk of *C. trachomatis* infection, since the alterations in the columnar epithelium facilitate the advance of the elementary bodies and hence the establishment of the infection(1,12,13). However, our patients with PMR or PDT did not showed mycosis, bacterial infections, or other diseases associated with PMR or PDT. Actually, the lack of a percise diagnosis in these patients prompted us to think that the infection by *C. trachomatis* could be responsible; however, the number of studied cases did not allow the establishment of a satisfactory conclusion.

On the other hand, the number of women with NP positive to *C. trachomatis* (3/50 Ag+, 8/50 IgG+ anti-*C. trachomatis*) makes it advisable to continue with studies of opportune diagnosis of the infection, and to follow up on these women, with the purpose of establishing the effective treatment to prevent the complications originated by the infection by *C. trachomatis*.

**REFERENCES.**

1.- Center for Disease Control and Prevention: Recomendations for the prevention and manegement of *Chlamydia trachomatis* infections. MMWR 1993; 42:1-41.


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