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Early Fetal Loss and Chlamydia Trachomatis Infection

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Abstract

Objectives: *Chlamydia trachomatis* infection is generally considered to be a risk factor to pregnancy. The aim of this study was to determine the role of *Chlamydia trachomatis* in miscarriage, as well as consider different diagnostic approaches.

Materials and methods: We collected serum, cervicovaginal swab specimens, and placental samples women with vaginal bleeding and/or abdominal pain in their first trimester of pregnancy. The presence of chlamydial infection was detected by conventional cultivation, by detection of chlamydial antigen using Polymerase Chain Reaction (PCR) and detection of IgG level using Immune-Enzymatic Assay (ELISA).

Results: Prevalence of miscarriage was significantly higher in group with positive cultivation of *C. trachomatis* infection (67.3% vs. 36.0%). We did not find a significant difference between the detection of chlamydial infection using conventional cultivation, ELISA or PCR. Association between a *C. trachomatis* positive diagnostic test and miscarriage remained significant (OR=2.41; 95% CI 1.32-3.35, p<0.01).

Conclusion: *C. trachomatis* infection is an important causative factor of miscarriage. *C. trachomatis* infection diagnostic procedures should be considered for further recommendations especially for women with recurrent fetal losses.

Keywords: Miscarriage; Chlamydia trachomatis; Diagnostic test

Introduction

Chlamydia trachomatis (*C. trachomatis*) infection is one of the most prevalent sexually transmitted diseases worldwide. The incidence of the *Chlamydia trachomatis* infection has dramatically increased during the past 10 years [1]. The World Health Organization estimates that there are 92 million new cases every year, with 10 million of these in Europe [2]. Unfortunately, more than 80% of cases are asymptomatic, particularly among females, and can lead to continued transmission of the infection and chronic infection with a high risk of pelvic inflammatory disease, ectopic pregnancy, chronic pelvic pain, salpingitis or tubal factor of infertility. *C. trachomatis* is a recognized agent of preterm labour and premature rupture of membranes; women are predisposed to postpartum pelvic inflammatory diseases and the neonatal complications of infant low birth, conjunctivitis and pneumonia [2,3]. Its role in miscarriage is unclear, but women after miscarriage, with unrecognised chlamydial infection, are at a higher risk of ascending infection [4].

C. trachomatis has been isolated in cervical swab, urine, or products of conception, but infection could be localized at deeper sites not amenable to sampling. Conventional culturing of *C. trachomatis* is technically difficult because of its intracellular life cycle. Even the molecular approaches can be difficult because of PCR inhibitors and the low number of copies. Hence, the development of a simple and reliable assay for the detection of *C. trachomatis* is essential.

The main purpose of this study was to investigate whether *C. trachomatis* is associated with miscarriage. We used conventional cultivation, molecular and serologic immunohistochemical approaches to compare the evidence of present *C. trachomatis* infection in women with or without miscarriage.

Materials and Methods

Between January 2009 and December 2011 we enrolled 316 women aged 17-35 years in first trimester of pregnancy at the Department of Obstetrics and Gynaecology, Jessenius Faculty of Medicine, Comenius University and University Hospital Martin (Martin, Slovakia). The study was approved by the local ethics committee. Pregnant women (pregnancy less than 12 weeks of gestation, based on ultrasound examination) admitted to department due to symptoms of vaginal bleeding and/or abdominal pain were included in to the study.

We collected demographic and obstetric data. Cervicovaginal swab specimens and serum were sampled at the time of admission to the department. Products of conception were collected in cases of miscarriage.

All patients who were included were tested for chlamydial infection by serological examination, determination of DNA by PCR and conventional cultivation of cervical smears and for women after abortion also tissue material from uterine cavity. Serum samples were tested for IgG specific anti-chlamydial antibodies level using enzyme-linked immunoabsorbent assay (MedacGmbH, Wedel, Germany).

Chlamydia antibody testing was performed according to the manufacturer's instructions and the test result was considered positive at an ELISA index of >1.1. Samples from cervicovaginal swabs and tissue from uterus were screened for *C. trachomatis* genome by using a RealArt™ Chlamydia trachomatis RG PCR Kit (Artus, Germany).

Women with positive cultivation were treated by Azithromycin, which is safe and recommended treatment during pregnancy.

We compared demographic data and risk factors of patients with and without miscarriage or *C. trachomatis* infection by the Pearson χ^2 test (or the Fisher exact test when indicated) for categorical variables.

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For continuous variables, medians were compared using the Wilcoxon-Mann-Whitney test and Student t-test. Statistical analyses were performed by using statistical software Medcalc 10.2, Mariakerke, Belgium. A p-value less than 0.05 were considered significant.

Result

Based on the result of cultivation test, we compared two groups, with (n=55) and without (n=261) positive cultivation of *C. trachomatis*. The women in the study were evaluated according to demographic variables: age, pregnancy week, pregnancy, parity, positive gynaecological history (abortion, surgery, inflammation, history of intrauterine device- IUD) (Table 1 and Figure 1).

From the whole study 131 (41.46%) pregnancies ended with miscarriage in the first trimester, from that 37 (11.7%) in the group with positive cultivation of *C. trachomatis*.

Logistic regression analysis revealed that women with positive cultivation on *C. trachomatis* had a higher risk of early fetal loss (OR=2.41; 95% CI 1.32-3.35, p<0.01) and also for preterm birth (OR=7.39; 95% CI 2.54-21.47, p<0.0005).

In term of investigative techniques, we did not detect a significant difference between the detection of chlamydial infection using the conventional cultivation, ELISA method and detection by PCR of material from the cervix or tissue from uterine cavity (Figure 2).

When we compared the results of cultivation tests to determine bacterial agents in relation to the early gestational losses, we found that the incidence of Group B Streptococcus(GBS) infection and Bacterial Vaginosis (BV) in pregnancy did not increase the risk of early fetal losses (OR 0.6, 95% CI 0.59-0.82, p=NS).

Discussion

Chlamydia trachomatis is a Gram-negative obligate intracellular bacterium with an incubation period of 7 to 21 days and a growth cycle of approximately 48 hours [5]. *C. trachomatis* has a worldwide distribution, affecting both sexes, but has a much greater impact on females than on males. It particularly affects young women and sexually active adolescents. Unfortunately, chlamydial infections may, in many cases, remain silent. Erythema, oedema, and mucopurulent discharge can be seen by physical examination during acute infection [5].

	Group A (n=131)	Group B (n=185)	P
Age (x, range)	23.9 (17-34)	24.5 (17-35)	NS
Gravidity (x ± SD)	1.8 ± 0.8	1.9 ± 0.8	NS
Parity (x ± SD)	0.5 ± 0.6	0.4 ± 0.6	NS
Positive gynaecology anamnesis (%)	19.5	15.1	NS
Gestation week (x ± SD)	8.9 ± 1.7	8.8 ± 1.7	NS

Table 1: Demo graphic variables.

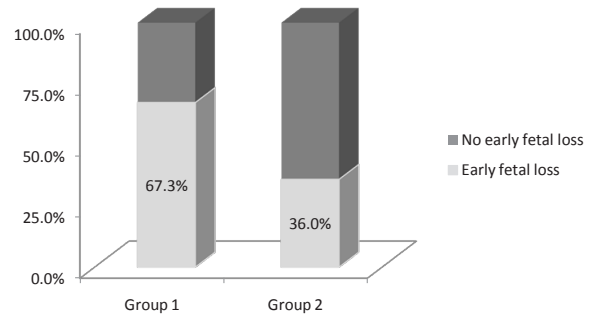

Figure 1: Prevalence of early fetal loss in group 1 with positive *C. trachomatis* cultivation test and group 2 with negative *C. trachomatis* cultivation test, p<0.0001.

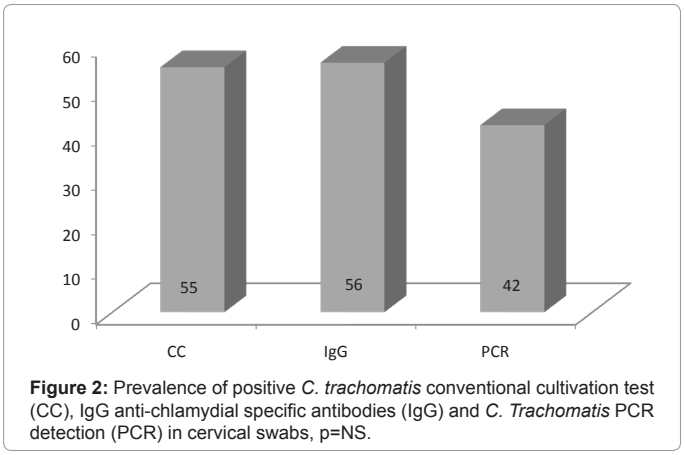

Figure 2: Prevalence of positive *C. trachomatis* conventional cultivation test (CC), IgG anti-chlamydial specific antibodies (IgG) and *C. Trachomatis* PCR detection (PCR) in cervical swabs, p=NS.

C. trachomatis serovars A through C infect mucosalepithelial cells in theconjunctivae and causetrachoma, the leading cause of infectious blindness worldwide. Serovars D through K infectmucosal epithelial cells in theurogenital tract and the leading cause of sexually transmitted bacterial infections in the United States and Europe. Serovars L1, L2, L2a, and L3 infect the genital epithelium as well as monocytes and cause a systemic disease called lymphogranuloma venereum [6].

During its unique developmental cycle, two different forms are observed: elementary bodies which are infectious but not able to divide, and reticulate bodies which are metabolically active and able to multiply [5]. The elementary body of *C. trachomatis* attaches to the epithelial cell surface and incorporates into phagosomes that migrate to the distal region of the Golgi complex. Lysosome fusion is prevented, and chlamydial infection averts immediate destruction. The elementary body then differentiates into the non-infectious but replicative reticulate body, which further divides by binary fission [6]. Although *C. trachomatis* can partially evade immune detection making infections fairly asymptomatic in many women, the infectious particles can be recognized by the host, with subsequent activation of host interferon-γ (IFN-γ) and pro-inflammatory cytokine secretion. In response to interferon exposure in vitro, reticulate body can enter a persistent and non-inflammatory state [6]. Chlamydial persistence has been described as a long-term association between chlamydiae and their host cells in which these bacteria remain in a viable but culture-negative state [1].

In pregnant women, *C. trachomatis* is even more critical as it may affect normal intra- and extra-uterine development. The pregnancy itself seems to increase the risk of *C. trachomatis* colonization and alter the immune response [2]. It may also influence the clinical manifestations of disease in pregnant women. The pathogenesis is still only partially understood. It is assumed that the chronic inflammatory response to chlamydia is modulated by the immune system with experimental evidence indicating that the bacterial heat-shock proteins (in particular HSP 60-kDa) reacting with human HSP are an important factor in the immune-pathogenesis of female genital inflammation [6,7].

The chlamydial HSP show an amazing analogy to human proteins. Thus, there should be a cross-reactivity between the human HSP60 and the bacterial cHSP60, which leads to the formation of antibodies against the HSP60 in the serum and follicular fluid of women exposed to *C. trachomatis*; these antibodies seems to have a negative impact on

embryonic growth, and increase the probability of adverse pregnancy outcomes [3,8]. Chlamydial HSP has also been found to induce trophoblast apoptosis by stimulating the toll-like receptor 4, which naturally mediates immune responses in placenta [3,9]. There also seems to be cross-reactivity between HSP10 and an embryonic protein, the early pregnancy factor, potentially causing miscarriage [10].

There is increasing evidence that *C. trachomatis* infections may result in adverse pregnancy outcomes in humans and animals. Also in our study, we found an association of spontaneous miscarriage with serologic and molecular evidence of *C. trachomatis* infection.

Several studies have failed to document an association between *C. trachomatis* and spontaneous or recurrent miscarriage [11-13]. However, these studies were conducted more than 15 years ago, before the recent increase in the prevalence of the *C. trachomatis* infection. Increased prevalence has improved statistical power. Sensibility and specificity of diagnostic methods have also improved during the past decade.

Recent studies have shown a relationship between miscarriage during the first trimester and the *C. trachomatis* infection by detection of anti-chlamydial IgG and IgA antibodies and endocervical swabs [14-16].

Baud et al. showed higher anti-*Chlamydia trachomatis* IgG prevalence in the miscarriage group of patient suffering from first trimester miscarriage (15.2%) than in controls (7.3%, $p<0.018$) [14]. The association between *Chlamydia trachomatis*-positive serology and miscarriage remained significant after adjustment for age, origin, education level, and number of sexual partners (OR 2.3, 95% CI 1.1-4.9). *Chlamydia trachomatis* DNA was more frequently amplified from products of conception or placentas taken from women who had suffered miscarriages (4%) than from controls (0.7%, $p<0.026$).

The study Wilkowska-Trojniel et al. examined 120 women in order to assess the frequency of *C. trachomatis* infections in patients with a history of miscarriage [15]. In a group of women with one spontaneous miscarriage, higher IgG specific antibodies were detected in serum by ELISA in 21.1% of the women, in women with two or more spontaneous miscarriage 36.4% and in the control group 4.4%. The study in this group included only patients in whom the cultivation tests excluded other infectious agents. This difference in our results may be due to multifactorial aetiology of recurrent fetal loss, with a stronger action of other factors such as exposure to other infectious agents that were not the exclusion criteria in our study.

In our study, we investigated other infectious aetiology of miscarriage (GBS and BV). One limitation of our study is the absence of the investigation of some viruses that cause chronic or recurrent maternal infection such as cytomegalovirus or parvoviruses. Among other bacterial infections, Ureaplasmaurealyticum, Mycoplasma hominis, Coxiellaburnettii or Brucellaabortus can also be associated with miscarriages.

The serologic association we observed is unlikely to be due to cross-reactivity with other chlamydial species such as C. abortus because we also observed a molecular association with miscarriage and C. abortus has been only infrequently associated with miscarriages in humans, mostly after zoonotic exposure.

C. trachomatis infection is highly prevalent among pregnant women. With regard to miscarriage associated with *C. trachomatis*, we showed a significantly increased risk in our population. Based on this result the significance of screening for this infection in pregnant women should be considered, especially when dealing with younger populations and risk factors for STDs. Thus, randomized clinical trials in treatment of *C. trachomatis* infection in pregnant women are needed to evaluate the possibility of reducing this risk [14,17].

Conclusion

The results of our study suggest that all women experiencing a miscarriage should be screened for *C. trachomatis* infection and, if positive, adequately treated to prevent recurrent fetal loss. Moreover, preconception screening might be proposed to reduce the prevalence of this adverse pregnancy outcome.

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