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The influence of *Chlamydia trachomatis* infection on spontaneous abortions

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ABSTRACT

Purpose: The aim of the study was to evaluate the frequency of *Chlamydia trachomatis* (*C.t.*) infection among women who experienced a miscarriage.
Materials and Methods: Patients referred to the Centre for STD Research and Diagnostics in Bialystok from the Department of Perinatology and from gynaecological outpatient clinics, after spontaneous abortion were enrolled in the study. *C.t.* infection diagnostics were performed among 76 women with 1 miscarriage and 44 patients with ≥2 miscarriages in anamnesis. Forty-six patients in the 2nd and the 3rd trimester of normal pregnancy served as a comparative group. Endocervical swabs as well as blood serum were obtained. To detect chlamydial DNA, direct PCR method was performed (Roche, Molecular Systems, N.J., USA). To detect IgA and IgG specific anti-chlamydial antibodies we used immunoenzymatic assay (medac, Hamburg, Germany).
Results: In patients with 1 miscarriage (gr.1), *C.t.* infection by means of PCR was detected in 11.8% of women (p=0.029), in patients with ≥2 miscarriages (gr.2) in 9.1% (p=0.198) and in the comparative group (gr.0) in 2.2%. Specific anti-chlamydial antibodies IgA class were detected in: 7.9 (p=0.082) in group 1, 4.5% (p=0.236) in group 2 and in 0% in group 0, and IgG class in 21.1% (p=0.024), 36.4% (p=0.000) and in 4.4%, respectively.
Conclusions: 1. *C.t.* infection is an important causative agent of miscarriages in women. 2. *C.t.* infection diagnostic procedures should be considered in screening tests during pregnancy.

Key words: *Chlamydia trachomatis*, miscarriage, recurrent spontaneous abortions, specific anti-chlamydial antibodies

INTRODUCTION

According to the Center for Disease Control and Prevention in Atlanta (CDC), urogenital infections caused by *Chlamydia trachomatis (C. trachomatis)* are the most prevalent sexually transmitted bacterial infectious diseases [1]. A distinctive feature of chlamydial urogenital infection is its asymptomatic or oligosymptomatic course. Earlier studies showed that bacterial chorioamnionitis can influence protease release, which leads to the rupture of the membranes, arachidonic acid cascade activation and uterine contractions. The result of these effects can be premature rupture of the membranes, preterm delivery or miscarriage [2,3]. Spontaneous abortions, despite the significant progress in gynecological diagnostic methods and advanced therapy strategies still remain important medical problem. Many authors tried to establish the relationship between *C. trachomatis* infection and pregnancy outcomes, and their results seem to be more reliable thanks to the development of the diagnostic methods, especially molecular ones. Early in vitro studies proved that the amniotic epithelium is susceptible to *C. trachomatis* infection and damage [4,5]. Thomas et al. further showed that foetal infection by *C. trachomatis* is also possible and can cause miscarriage [3]. The authors determined the presence of *C. trachomatis* in the uterine cervix and the amniotic fluid using direct fluorescence method (DFA) in women with premature rupture of the membranes.

Schwarz et al. studies demonstrated that lysosomes of the foetal membranes' cells contain a high concentration of A2 phospholipase which is a precursor in prostaglandin synthesis [6]. *C. trachomatis* infection by damaging the amniotic or deciduous cells' lysosomes stimulates prostaglandin synthesis, which causes uterine contractions leading to miscarriage or preterm delivery. In Polish literature, the issue concerning the relationship of *C. trachomatis* infection with abortions is seldom raised.

The aim of the study was to evaluate the frequency of *C. trachomatis* infection among women who experienced miscarriage.

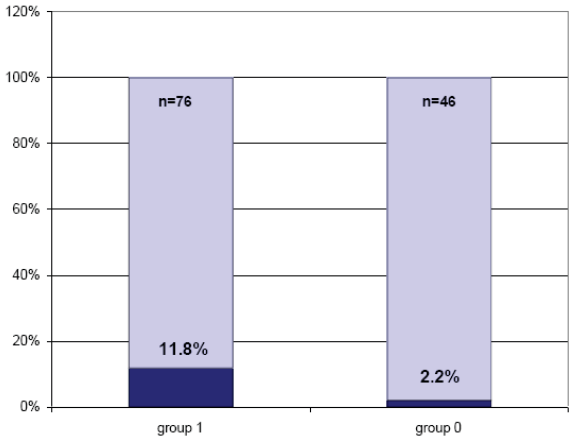
MATERIAL AND METHODS

We enrolled 120 women aged 19-44 years with miscarriages in the past, referred to the Centre for Sexually Transmitted Diseases Research and Diagnostics in Bialystok, from gynaecological outpatient clinics and patients from the Department of Perinatology of the Medical University of Bialystok for *C. trachomatis* tests. The study was conducted in the years 2003-2006. All the women gave their consent to participate in the study. The time interval between miscarriage and testing ranged from one to six months. Most patients came 4-6 months after miscarriage. Gestation age at the time of miscarriage ranged between 8 and 12 weeks. All the patients stated not to have had any antibiotic treatment during the last 3 months. Those women in whom bleeding was present were excluded. All the women enrolled into the study had had tests performed to rule out other than *C. trachomatis* infectious agents, such as Treponema pallidum, Toxoplasma gondii as well as other genital infections, including bacterial vaginosis, Trichomonas vaginitis, candidiasis.

In group 1, we examined 76 women aged 19-42 years old (mean age – 28.4 years), who experienced spontaneous abortion once and in group 2, we examined 44 patients with repeated abortion (two or more). In group 2, twofold abortion in the past took place in 25 women, threefold in 10 and fourfold in 9 patients. In this group patients were 19-44 years old (mean age 31.05 years). The comparative group (group 0) included 46 women, in the II and III trimester of normal uncomplicated pregnancy, with no history of pregnancy failure and with no complaints or urogenital disorders.

Endocervical swabs as well as serum samples were obtained. Polymerase chain reaction (PCR, Roche Molecular Systems, N.J., USA) which is a direct method was performed to detect *C. trachomatis* genetic material in cervical swabs. To detect IgA and IgG specific anti-chlamydial antibodies in serum samples we used immunoenzymatic assay (p-ELISA, medac, Hamburg, Germany). Chemically defined synthetic peptide from the immunodominant, variable domain IV of the *C. trachomatis* major outer membrane protein (MOMP) was applied as antigen in kits. Diagnostic procedures were performed at the Chlamydial Laboratory of the Centre for STD Research and Diagnostics in Bialystok.

Figure 1. **Prevalence of *C. trachomatis* detection in cervical swabs (PCR) in women who experienced 1 abortion (group 1) and in the comparative group (group 0). p=0.029 (test chi² Pearson)**



Data obtained in the study were statistically analysed by Chi²Pearson and Fisher test methods. Statistical value was α=0.05. We used Statistica PL program (StatSoft). This study was approved by the University of Bialystok Ethics Committee.

RESULTS

In group 1 (patients with 1 spontaneous abortion), *C. trachomatis* infection by direct method was detected in 9/76 patients (11.8%). In the comparative group infection was detected in 1/46 patient (2.2%) and the statistical difference was significant (p=0.029) (*Fig. 1*).

IgA specific anti-chlamydial antibodies were detected in 6/76 patients, and IgG in 16/76 patients, which is 7.9% and 21.1% respectively. In the comparative group, IgG antibodies were detected in 2/46 (4.4%) and the difference was statistically significant (p=0.024) in contrast to IgA antibodies (p=0.082) (*Fig. 2, 3*).

In group 2, *C. trachomatis* infection by PCR method was confirmed in 4/44 of cases (9.1%). In the comparative group, direct method revealed only one case of cervical infection (2.2%) (*Fig. 4*). Statistical analysis of direct studies in the group with ≥2 miscarriages was not statistically significant (p=0.198). Specific anti-chlamydial antibodies IgA in this group were detected in 2/44 of women (4.5%). IgG antibodies for *C. trachomatis* were present in 16/44 of patients (36.4%). In group 0, IgA antibodies in serum were absent and IgG antibodies were present in 2/46 of women (4.4%). In reference to the comparative group, significant results were obtained only for IgG antibodies (p=0.000) (IgA – p=0.236) (*Fig. 5, 6*).

We were trying to establish the frequency of *C. trachomatis* infection in women with the history of recurrent abortion by direct and serological methods.

C. trachomatis infection by direct method was detected in 9/76 women (11.8%) with one previous miscarriage,

Figure 2. **Prevalence of anti-chlamydial specific antibodies detection IgA in women with 1 previous abortion (group 1) and in the comparative group (group 0). p=0.082 (Fisher's test)**

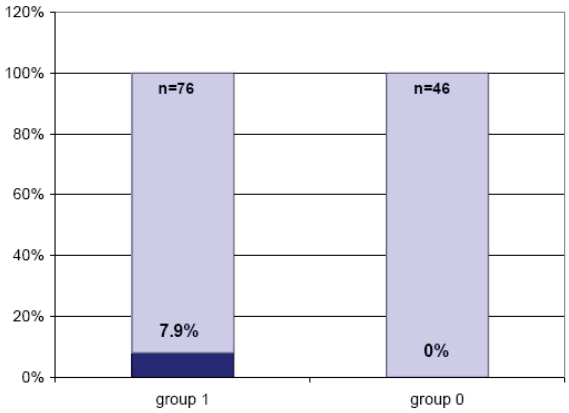


Figure 4. **Prevalence of *C.trachomatis* detection in cervical swabs (PCR) in women who experienced 2 abortions (group 2) and in the comparative group (group 0). p=0.198 (Fisher's test)**

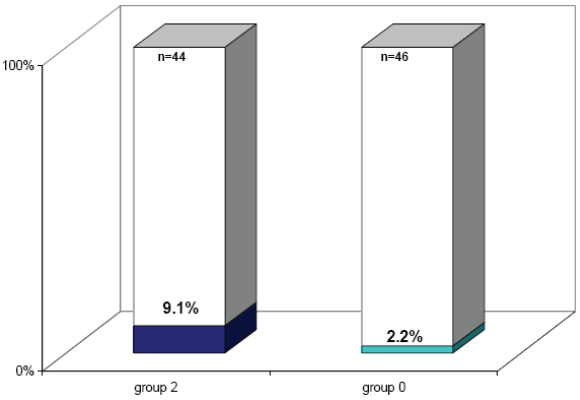
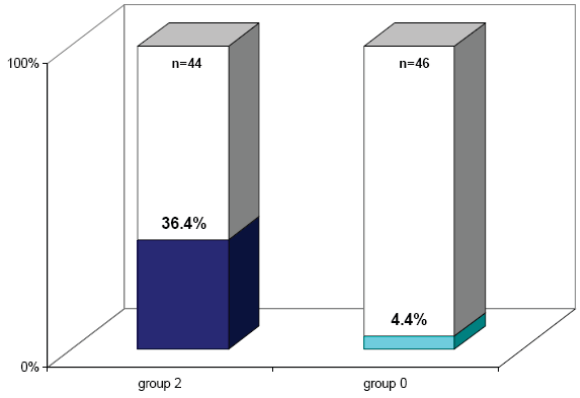


Figure 6. **Prevalence of anti-chlamydial specific antibodies detection IgG in women with 2 previous abortions (group 2) and in the comparative group (group 0). p=0.000 (test chi2 Pearson)**



in 2/25 (8%) with two miscarriages and in 2/19 of patients (10.5%) with three or more previous abortions. Comparing all these groups, the difference appeared not to be statistically significant (chi^2 Pearson: p= 0.865) *(Tab. 1)*.

Serological tests for IgA specific anti-chlamydial antibodies revealed 6 out of 76 positive cases in women with 1 previous abortion (7.9%), 1/25 (4%) cases with 2 miscarriages and in 1/19 (5.3%) with habitual abortions. Statistical analysis of the results of IgA antibodies in women with a different number of previous abortions did not show significance (p=0.767; p=0.559) *(Tab. 2)*.

Figure 3. **Prevalence of anti-chlamydial specific antibodies detection IgG in women with 1 previous abortion (group 1) and in the comparative group (group 0). p=0.024 (test chi^2 Pearson)**

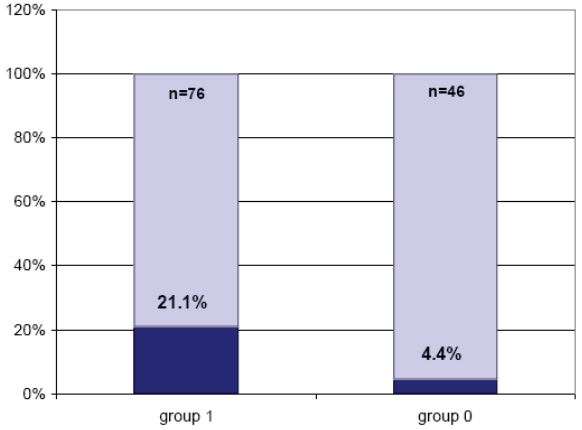


Figure 5. **Prevalence of anti-chlamydial specific antibodies detection IgA in women with 2 previous abortions (group 2) and in the comparative group (group 0). p=0.236 (Fisher's test)**

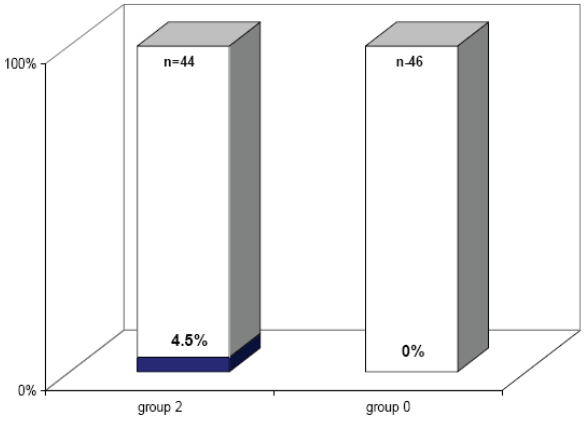


Table 1. **Prevalence of *C. trachomatis* detection in cervical swabs in women with habitual abortions.**

Number of earlier miscarriages	PCR method				Total
	(+)		(-)		
	n	%	n	%	
1	9	11.8	67	88.2	76
2	2	8.0	23	92.0	25
≥3	2	10.5	17	89.5	19
Total	13	10.8	107	89.2	120
p*					0.865
p***					0.747

*- test chi^2 Pearson
***test chi^2 for trend

Table 2. **Prevalence of anti-chlamydial specific antibodies detection IgA in women with habitual abortions.**

number of earlier miscarriages	specific anti-chlamydial antibodies IgA				Total
	(+)		(-)		
	n	%	n	%	
1	6	7.9	70	92.1	76
2	1	4.0	24	96.0	25
≥3	1	5.3	18	94.7	19
Total	8	6.7	112	93.3	120
p*					0.767
p***					0.559

*- test chi^2 Pearson
***test chi^2 for trend

Table 3. **Prevalence of anti-chlamydial specific antibodies detection IgG in women with habitual abortions.**

number of earlier miscarriages	specific anti-chlamydial antibodies IgG				Total
	(+)		(-)		
	n	%	n	%	
1	16	21.1	60	78.9	76
2	8	32.0	17	68.0	25
≥3	8	42.1	11	57.9	19
Total	32	26.7	88	73.3	120
p*					0.142
p***					**0.048**

*- test chi^2 Pearson
***test chi^2 for trend

The frequency of IgG specific antibodies for *C. trachomatis* detection appeared to be directly proportional to the number of previous miscarriages. In the group with one miscarriage there was 16/76 of positive cases (21.1%), whereas in the group with 2 abortions we detected IgG anti-chlamydial antibodies in 8/25 (32%), and in women with habitual abortions in 8/19 of cases (42.1%), which was statistically significant *(Tab. 3)*.

DISCUSSION

Our study showed the presence of genitourinary tract *C. trachomatis* infection in 11.8% of patients with one miscarriage (group 1) and in 9.1% of women with two or more miscarriages (group 2). Anti–chlamydial IgA and IgG antibodies were found respectively in 7.9% and 21.1% of cases in group 1, and in 4.5% and 36.4% in group 2.

Higher percentage of positive direct results was established by Elias et al. [7] who confirmed cervical chlamydial infection in women with imminent abortion in 23/87 (26.4%).

In Polish literature the question of bacterial pathogens influence on pregnancy outcome was brought up by Dudkiewicz et al. [8]. Among 144 women who experienced miscarriage, *C. trachomatis* was confirmed the most frequently comparing to other bacterial pathogens isolated from the cervix (18.7%), while E. coli, Klebsiella or Staphylococcus haemolyticus were detected in 9.2%, 7.6% and 7.6%, respectively. The prevalence of *C. trachomatis* infection in adverse pregnancy outcome was significant (48.6%). Choroszy-Król et al. showed *C. trachomatis* infection in cervical swabs by direct immunofluorescence assay in 44.4% of women with previous miscarriages [9]. Zgórniak-Nowosielska et al. based on the culture method, confirmed cervical chlamydial infection in 80/344 (23%) of women who experienced abortion [10].

In our study, we tried to establish the role of *C. trachomatis* infection in recurrent abortions. More frequently, we detected specific IgG anti-chlamydial antibodies among women with previous miscarriages in comparison to IgA antibody tests results as well as direct detection of the pathogen. We determined a correlation between the number of experienced spontaneous miscarriages and the frequency of IgG specific anti-chlamydial antibodies detection. In cases with 1 miscarriage, chlamydial antibodies were detected in 16/76 of patients (21.1%), in cases with 2 abortions in 32% and in 3 and 4 abortions in 42.1%. There was not a similar correlation observed between the number of miscarriages and study results determined by PCR method or IgA specific antibody detection. *C. trachomatis* infection by PCR method was confirmed the most often in the group with one previous abortion (11.8%), which was statistically significant compared to the comparative group (p=0.029). The patients came to the Centre 4-6 months after spontaneous abortion. This may have had an effect on the higher detection rate of IgG antibodies as compared to class IgA antibodies.

Quinn et al. found IgG antibodies to *C. trachomatis* in 57.6% of women with more than one spontaneous abortion in the past more frequently compared to patients with uncomplicated pregnancy (33.7%) [11]. Witkin et al. also confirmed the relationship between the presence of anti-chlamydial antibodies in serum and recurrent abortions [12]. Chlamydial infection by means of serological methods (IgG specific antibodies) was detected in their study in 6/47 (12.8%) of women with 1 previous miscarriage, in 4/33 (12.1%) with 2 miscarriages in the past, and in 7/17 (41%) with 3 miscarriages in the past and in as many as 60% (6/10) of patients with 4 miscarriages. Additionally, the authors observed that the higher titers of specific antibodies correlated with recurrent abortions. Nevertheless, Rae et al. did not confirm such an association, as anti-chlamydial IgG antibody tests revealed no significant differences between patients with recurrent abortions (26/106 – 24.5%) and the control group (20.3%) [13]. Paukku et al. also described the lack of correlation between serum antibodies to *C. trachomatis* and recurrent pregnancy loss [14]. Antibodies were detected in women who experienced abortions as often as in those in the control group and as often as in women with 1 or 2 abortions in the past. IgG anti-chlamydial antibodies were also detected with similar frequency in the study group and the comparative group in a study conducted by Osser's

et al. In women who experienced miscarriage, *C. trachomatis* infection was confirmed in 137/349 (39.3%) and in 116/349 (33.2%) respectively [15]. Similarly, Sozio et al. also did not support the relationship between cervical chlamydial infection and the development of spontaneous abortion. The infection was detected by LCR method in women with abortion in anamnesis more rarely than in the control group (in 3.8% and 8.5% respectively) [16].

Based on our study results, it is very difficult to establish the exact and undeniable role of chlamydial infections in miscarriages. However our results showed that *C. trachomatis* infection has to be taken into consideration as far as pregnancy failures are concerned. Serology methods, based on specific anti-chlamydial antibodies detection seem to be important in diagnosing *C. trachomatis* infections. Further studies should be conducted on a larger number of patients.

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