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Original Article

Chlamydia trachomatis infection in infertile and pregnant women in southern Brazil

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ABSTRACT

Introduction: *Chlamydia trachomatis* (CT) is the most prevalent sexually transmitted bacterial infection, affecting mainly young, sexually active women. Untreated infection may lead to reproductive complications due to tubal damage. Infections during pregnancy may cause preterm labor, low birth weight, perinatal death, and neonatal conjunctivitis and pneumonia. There are few data on CT infection in Brazil. The aim of this study was to determine CT prevalence in infertile and pregnant women.

Methods: A cross-sectional study included 77 infertile and 60 asymptomatic pregnant women. First-void urine was tested for CT using PCR (Polymerase Chain Reaction). Blood samples were collected for CT IgG antibodies testing using indirect immunofluorescence. A questionnaire about medical, gynecological, and sexual history was completed by all participants.

Results: We found statistically similar prevalence of PCR and IgG antibodies between the groups. There was a 61% prevalence of CT IgG antibodies in infertile women and 56.7% in pregnant women. PCR was positive in only one (1.3%) infertile woman and in none pregnant women.

Conclusion: There is a high prevalence of CT IgG antibody in Brazilian pregnant and infertile women, but we found a low prevalence of positive PCR in the urine samples. CT antibodies were associated with sexual behavior and smoking.

Keywords: *Chlamydia trachomatis; Chlamydia infections; prevalence; nucleic acid amplification techniques; infertility; female; fluorescent antibody technique.*

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Chlamydia trachomatis (CT) is the most prevalent sexually transmitted bacterial infection[1]. According to the World Health Organization (WHO), 131 million people were infected worldwide in 2012[2]. It affects mainly young, sexually active women[3]. Most infections with CT are asymptomatic[4]. In women, untreated infection may lead to pelvic inflammatory disease (PID) with the risk of serious reproductive complications, such as chronic pelvic pain, tubal factor infertility (TFI), and ectopic pregnancy[5]. Studies suggest that chlamydial infection in pregnant women can enhance the risk of preterm labor, low birth weight, and perinatal death[6]. Additionally, CT vertical transmission usually causes neonatal inclusion conjunctivitis and/or pneumonia[7].

Among various methods available for CT infection diagnosis, nucleic acid amplification tests (NAATs) are preferable due to their high sensibility and specificity and because they can be performed using non-invasive samples, such as urine and vaginal swabs[8]. *Chlamydia* immunoglobulin (Ig) G antibodies persist for years even after antibiotic treatment and are used as markers of a past infiltrating CT infection[9].

In Brazil, since routine screening is not recommended by the public health system, there are few data on CT infection. The purpose of this study was to estimate the prevalence of CT infection in infertile and pregnant women seen at a public hospital in Southern Brazil.

METHODS

Subjects

A cross-sectional study in infertile and pregnant women seen at Hospital de Clínicas de Porto Alegre (HCPA) was conducted from January to December 2015. Participants were divided in two groups. The infertile group (n=77) included women being treated at the infertility outpatient clinic of HCPA. They were unable to conceive after 1 year of regular unprotected sexual intercourse. The pregnant group (n=60) included pregnant asymptomatic women of any gestational age. Exclusion criteria were similar for both groups: acute symptoms of PID, use of antibiotics during the last 30 days, age under 18 years old, and refusal to participate in the study. First-void urine (FVU) for CT "in house" Polymerase Chain Reaction (PCR) test and a single venous blood sample for indirect immunofluorescence (IIF) for CT serological testing were collected from all participating women. Additionally, all women answered a questionnaire about their sexual and gynecological medical history.

Laboratory Methods

Antibody testing was performed by IIF in blood samples at the Hospital de Clínicas de Porto Alegre laboratory. The commercial kit Viro-Immun (VIRO-IMMUN Labor-Diagnostika GmbH Oberursel /Germany) was used. FVU samples were immediately shipped to Amplicon laboratory, and PCR was performed using an "in house" method developed according to previous studies[10,11].

Ethical Aspects

The study was approved by the Research Ethics Committee of the Hospital de Clínicas de Porto Alegre, and all patients were informed about this study through the informed consent form.

Statistical Analysis

The sample size was calculated using the WinPEPI program (*Programs for Epidemiologists for Windows*) 11.43 version and was based on previous findings of IgG prevalence (39% in infertile women and 19% in pregnant women)[12]. Considering a 95% confidence interval and a statistical power of 71.6%, we included n=77 infertile and n=60 pregnant participants. Data processing and analyses were performed using the SPSS 21.0 (SPSS, Chicago, Ill, USA). Initially, a descriptive analysis of the main characteristics of the participants and their related risk factors was performed. Quantitative variables were expressed as mean and standard deviation (mean±SD) or median value and interquartile amplitude (median±IQ). Categorical variables were expressed as absolute and relative frequencies. We used the t-student test to compare quantitative variables. The Mann-Whitney U test was used when data distribution was asymmetrical. Pearson's chi-squared test or Fisher's exact test were applied to sets of categorical data. We also calculated IgG's sensitivity, specificity, positive and negative predictive value, the accuracy and the odds ratio to predict TFI. $P \leq 0.05$ was considered statistically significant.

RESULTS

FVU and serum samples of 60 healthy pregnant women and 77 infertile women were investigated. Pregnant women were younger and had lower rates of steady sexual partner, previous PID, and previous pelvic surgery than the infertile group. They also had higher rates of regular condom use. The characteristics of the sample are summarized in Table 1. The mean gestational age was 30.6 weeks (± 7.4) among pregnant women. In the infertile group, median time of infertility was 6 years (3-10). Primary infertility was found in 84.4% of women and 54% of them had tubal damage. Sixteen women (20.8%) suffered from more than one cause of infertility (table 2). We found statistically similar prevalence rates of PCR and IgG antibodies between the groups. However,

infertile women had higher median of IgG titration (table 3). When we compared the TFI subgroup with controls, there were statistically more individuals with high titration (IgG≥128) levels among infertile women (table 4). Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), accuracy, and odds ratio were calculated concerning IgG's ability to predict tubal damage (table 5). We found association between positive IgG antibody test and higher number of sexual partners in life (table 6). IgG high titration level (IgG≥128) was associated with younger age of first sexual intercourse, higher number of sexual partners and smoking (table 7). Individuals with TFI were more likely to have previous history of PID than women with other causes of infertility (table 8).

Table 1: Sample characteristics.

Variables	Infertile (n=77)	Pregnant (n=60)	p
Age (years) – mean±SD	32.8 ± 4.4	27.1 ± 6.4	<0.001
Number of pregnancies – median (P25 – P75)	0 (0-1)	2 (1-3)	<0.001
Previous abortion – n(%)	20 (26.0)	13 (21.7)	0.701
Age of first sexual intercourse (years) – mean±SD	16.4 ± 2.3	16.3 ± 1.9	0.710
Number of sexual partners – median (P25 – P75)	3 (1-4.5)	3 (2-5)	0.488
Steady sexual partner – n(%)	77 (100)	55 (91.7)	0.015
Regular condom use – n(%)	4 (5.2)	15 (25.0)	0.002
Previous PID – n(%)	25 (32.5)	2 (3.3)	<0.001
Previous ectopic pregnancy – n(%)	7 (9.1)	1 (1.7)	0.079
Previous pelvic surgery – n(%)	33 (42.9)	3 (5.0)	<0.001
Previous HPV infection – n(%)	12 (15.6)	4 (6.7)	0.179
Smoking – n(%)	10 (13.0)	4 (6.7)	0.354

SD = standard deviation; PID = pelvic inflammatory disease; HPV = human papilloma virus.

Table 2: Characteristics of the infertile group.

Variables	Infertile (n=77)
Previous empirical treatment – n(%)	33 (42.9)
Primary infertility – n(%)	65 (84.4)
Time of infertility (years) – median (P25 – P75)	6 (3-10)
Causes of infertility – n(%)	
Tubal damage	42 (54.5)
Other causes	47 (61.0)
Anovulation	11 (14.3)
Endometriosis	15 (19.5)
Male	20 (26.0)
Uterine	1 (1.3)
Multiple causes – n(%)	16 (20.8)

Table 3: Prevalence of CT IgG and PCR in groups.

CT test	Infertile (n=77)	Pregnant (n=60)	p
IgG- n(%)			0.733
Positive	47 (61.0)	34 (56.7)	
Negative	30 (39.0)	26 (43.3)	
IgG titration– median (P25 -P75)	256 (128-512)	128 (64-256)	0.016
IgG ≥128 – n(%)	39 (50.6)	19 (31.7)	0.040
PCR			1.000
Positive	1 (1.3)	0 (0.0)	
Negative	76 (98.7)	60 (100)	

IgG = immunoglobulin G; PCR = Polymerase Chain Reaction.

Table 4: Prevalence of CT IgG and PCR in the tubal factor infertility subgroup and in pregnant women.

CT test	TFI infertility (n=42)	Pregnant (n=60)	p
IgG			0.571
Positive	27 (64.3)	34 (56.7)	
Negative	15 (35.7)	26 (43.3)	
IgG titration – median (P25 – P75)	256 (128-512)	128 (64-256)	0.002
IgG ≥128 – n(%)	26 (61.9)	19 (31.7)	0.005
PCR			-
Positive	0 (0.0)	0 (0.0)	
Negative	42 (100)	60 (100)	

TFI = tubal factor infertility; IgG = immunoglobulin G; PCR = Polymerase Chain Reaction.

Table 5: IgG diagnostic properties in predicting tubal damage.

Diagnostic properties	Positive (>0)	IgG ≥ 128	IgG ≥ 256
Sensitivity	64.3%	61.9%	47.6%
Specificity	42.9%	62.9%	71.4%
PPV	57.4%	66.7%	66.7%
NPV	50.0%	57.9%	53.2%
Accuracy	54.5%	62.3%	58.4%
Odds ratio	1.35	2.75	2.27

PPV = positive predictive value; NPV = negative predictive value.

Table 6: Association between participants' characteristics and IgG positivity.

Variables*	IgG Positive (IgG >0) (n=81)	IgG Negative (IgG=0) (n=56)	p
Age	30.6 ± 5.7	29.8 ± 6.5	0.423
Age of first sexual intercourse	16.1 ± 2.1	16.8 ± 2.0	0.060
Number of sexual partners	3 (2-5)	2 (1-4)	0.036
Steady sexual partner	77 (95.1)	55 (98.2)	0.648
Condom use	13 (16.0)	6 (10.7)	0.524
Previous PID	17 (21.0)	10 (17.9)	0.815
Previous ectopic pregnancy	6 (7.4)	2 (3,6)	0.471
Previous pelvic surgery	26 (32.1)	10 (17.9)	0.096
Previous HPV infection	11 (13.6)	5 (8.9)	0.574
Smoking	11 (13.6)	3 (5.4)	0.202
Number of pregnancies	1 (0-2)	1 (0-2)	0.186

* Expressed as mean±SD, median (percentile 25-75) or n(%). PID = pelvic inflammatory disease; HPV = human papilloma virus.

Table 7: Association between participants' characteristics and IgG titration.

Variables*	High titration (IgG ≥ 128) (n=58)	Low titration (IgG<128) (n=79)	p
Age	30.5 ± 5.3	30.1 ± 6.6	0.743
Age of first sexual intercourse	15.7 ± 1.9	16.8 ± 2.2	0.002
Number of sexual partners	3.5 (2-7)	2 (1-4)	0.007
Steady sexual partner	54 (93.1)	78 (98.7)	0.162
Condom use	10 (17.2)	9 (11.4)	0.466
Previous PID	15 (25.9)	12 (15.2)	0.182
Previous ectopic pregnancy	6 (10.3)	2 (2.5)	0.071
Previous pelvic surgery	20 (34.5)	16 (20.3)	0.094
Previous HPV infection	8 (13.8)	8 (10.1)	0.696
Smoking	10 (17.2)	4 (5.1)	0.041
Number of pregnancies	1 (0-2)	1 (0-2)	0.912

* Expressed as mean±SD, median (percentile 25-75) or n (%). PID = pelvic inflammatory disease; HPV = human papilloma virus.

Table 8: Association between participants' characteristics and tubal factor infertility.

Variables*	Tubal factor infertility (n=42)	Other causes of infertility (n=35)	p
Age	32.7 ± 4.1	32.9 ± 4.7	0.825
Number of pregnancies	0 (0-2)	0 (0-1)	0.094
Age of first sexual intercourse	16.2 ± 1.7	16.7 ± 2.8	0.347
Number of sexual partners	3 (1-5)	3 (1-4)	0.354
Condom use	2 (4.8)	2 (5.7)	1.000
Previous PID	22 (52.4)	3 (8.6)	<0.001
Previous ectopic pregnancy	6 (14.3)	1 (2.9)	0.119
Previous pelvic surgery	20 (47.6)	13 (37.1)	0.488
Previous HPV infection	8 (19.0)	4 (11.4)	0.547
Smoking	5 (11.9)	5 (14.3)	1.000

* Expressed as mean±SD, median (percentile 25-75) or n (%). PID = pelvic inflammatory disease; HPV = human papilloma virus.

DISCUSSION

Using different diagnostic methods, we found no significant differences between fertile and infertile women for CT infection. This study found a 61% prevalence of CT IgG antibodies in infertile women and 56.7% in pregnant women. A study conducted in India[13] found a 68% prevalence of CT IgG antibodies in infertile women and 10% in healthy pregnant women. Siemer et al.[12] also showed statistically different prevalence rates of CT IgG between infertile and pregnant women (39% vs. 19%, respectively). Rashidi et al.[14], using ELISA to test IgG antibodies, observed lower rates of seroprevalence and found no difference between infertile and pregnant women (9% vs. 5%, respectively). In our study, the high prevalence of CT antibodies in pregnant women may be caused by cross-reaction with *C. pneumoniae,* because most serological tests are not species-specific tests.

PCR in FVU was positive in only one (1.3%) infertile woman and in none of the pregnant participants. Previous studies in Brazil have found variable prevalence rates of CT using NAATs. Ramos et al.[15] tested 161 women between 15 and 44 years old in Porto Alegre-RS and found a 0.59% CT prevalence in urine samples. Two multicenter studies were conducted in Brazil to estimate CT prevalence in pregnancy and found a 9.8% prevalence when FVU was used to perform PCR analysis, whereas a 9.4% prevalence was found when hybrid capture was performed in endocervical swabs[16,17].

Some authors believe that CT IgG antibodies are as accurate as hysterosalpingography (HSG) in predicting TFI[18]. In our study, IgG titration of 128 had better diagnostic properties in predicting tubal damage. Nevertheless, our findings regarding IgG were worse than previous studies. Malik et al.[19] found 72.7% sensitivity, 80% PPV, 77.7% specificity, and 70% NPV of IgG antibodies measured by ELISA. A meta-analysis performed in 2008 indicated that the predictive value of CT antibody test for tubal pathology is limited: its sensitivity varies between 30% and 88%, whereas its specificity varies between 45% and 100%[20].

We found correlation between IgG presence and younger age of first sexual intercourse, higher number of sexual partners, and smoking. This finding agrees with previous studies. Datta et al.[21] concluded that age under 25 years old, multiple sexual partners, irregular use of condom, and previous history of any sexual transmitted disease are risk factors for CT infection.

A limitation of our study is that pregnant women were tested mostly in the third quarter of pregnancy. Therefore, we could not differentiate infections that occurred previously or during pregnancy. Besides that, the low prevalence of positive PCR in infertile women could be explained by the fact that almost 43% of them had documented previous empirical treatment with azithromycin during infertility investigation. Furthermore, CT may persist in a viable and metabolically active state in the upper genital tract, despite negative PCR results in urine or endocervical samples[22]. In addition, we have to consider that the mean age in our study population was relatively high, and more than 91% of women in our population had a steady sexual partner. Because most CT infections occur in people younger than 25 years old who are sexually promiscuous, the high seroprevalence of CT and the low rates of positive PCR found in our study are understandable.

In conclusion, we demonstrated a statistically similar high prevalence of CT IgG antibody in Brazilian pregnant and infertile women, but a low prevalence of positive PCR in urine samples. CT antibodies were associated with sexual behavior and smoking.

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