

## Effects of *Chlamydia trachomatis* Infection on Fertility; A Case-Control Study

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### Abstract

**Background:** Nowadays, *Chlamydia trachomatis* is known as a causative agent of infertility. Because of, asymptomatic nature of infection, many may suffer from its lasting complications such as infertility. This study was performed in Tehran during April 2007 to April 2008 to compare the prevalence of *Chlamydia trachomatis* infection in fertile and infertile women using ELISA and PCR methods.

**Methods:** Overall, 234 infertile and 223 pregnant women, as the fertile group, participated in this hospital-based case-control study. After completing an informed consent form and the questionnaire, first catch urine and blood sample were obtained for PCR and ELISA (IgG, IgM) tests, respectively. Logistic regression analysis was used to control possible confounding factors, and determine adjusted odds ratio of infertility due to the infection.

**Results:** PCR results revealed that 29 (12.4%) of the infertile and 19 (8.5%) of the fertile women were positive for *C. trachomatis* infection ( $p=0.440$ ). IgG was positive in 21 (9.0%) of the infertile and 11 (5.0%) in the fertile group ( $p=0.093$ ). IgM assays identified that 2 (0.9%) of the infertile and 4 (1.8%) of the fertile women were positive for the micro-organism ( $p=0.375$ ).

**Conclusion:** We found no significant differences among fertile and infertile women for *Chlamydia trachomatis* infection. Nevertheless, molecular techniques which are more sensitive, more specific and non-invasive can be used to detect *C. trachomatis* infection.

**Keywords:** Case control study, *Chlamydia trachomatis*, Enzyme-linked immunosorbent assay, Infertility, Polymerase chain reaction.

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### Introduction

**C**hlamydia *trachomatis* is the most common sexually transmitted bacterial infection (1). More than 900.000 infections with *C. trachomatis* were reported and twice as many were

unreported in the United States during 2004 (2). Vaginal discharge, dysuria, postcoital bleeding, intermenstrual bleeding and abdominal pain are some of the symptoms that are associated with

genital infection with *C. trachomatis* in women (3). This type of bacterial infection may be asymptomatic and delay in its diagnosis may cause harmful effects but early detection and appropriate treatment can minimize complications in the patients. Therefore, it is suggested that countries provide large scale screening programs for at risk patients (4). Screening needs to establish accurate and cost-effective tools and laboratory tests. Polymerase chain reaction (PCR) has been successfully used in research studies for the detection of *Chlamydia trachomatis* (CT) DNA. PCR is more sensitive than high quality cultures (5). This test is expensive and needs more time but a previous study suggests that it is a promising method for the detection of asymptomatic pelvic infection in patients with unexplained infertility (6). Enzyme linked immunosorbant assay (ELISA) can detect *C. trachomatis* antibodies as another diagnostic tool (7).

Infection with *C. trachomatis* can cause urethritis, cervicitis, adnexitis, pelvic inflammatory disease, or ectopic pregnancy (8) Subfertility (9) and infertility (10) in women. Most of these patients are asymptomatic and are usually diagnosed with the infection when they undergo infertility diagnostic procedures (11). In some studies *C. trachomatis* had greater prevalence in infertile than fertile women (12), but their prevalence was shown to be the same in other studies (10, 11).

A previous study reported that the molecular prevalence of *C. trachomatis* was 12.6% in woman in Tehran, the capital of Iran (13), and in another study it was 21.25% in women attending Shahid Beheshti Hospital in Isfahan, Iran. Considering the different prevalence rates of *C. trachomatis* infection in Iran, it is vitally essential to assess the impact of *C. trachomatis* on the reproductive health of women (14). Most studies in Iran have been limited to case-series but case-control studies are so limited. The comparative prevalence of *Chlamydia* is also one of the questions to be answered in both fertile and infertile women.

We compare the prevalence of *C. trachomatis* infection in fertile and infertile women with both PCR and ELISA methods in Tehran, Iran, during April 2007 to April 2008.

We undertook this study as there seemed to be limited studies on *C. trachomatis* prevalence in infertile patients in Iran.

## Methods

**Setting:** This case-control study was performed at the infertility clinic, prenatal clinic and delivery room of Vali-e-Asr Hospital, one of the central public hospitals of Tehran University of Medical Sciences, Tehran, Iran from April 2007 to April 2008.

The study was approved by the Ethic Committee of Tehran University of Medical Sciences.

Consecutive sampling was used. After signing an informed consent, each participant completed a questionnaire on demographic characteristics including age, education, occupation, gravidity, contraception and previous parities. Information about infertility duration, type and etiology of infertility, history of abdominal surgeries, abortion or ectopic pregnancy were also obtained and a physical examination was conducted by a gynecologist. Up to 15 ml of first catch urine (after not voiding urine for at least 2 hr) was collected into sterile containers without preservatives. The specimens were transported at room temperature for urine processing. Blood samples were obtained by venopuncture and the serum was separated by centrifugation and stored at  $-20^{\circ}\text{C}$ .

**Participants:** Calculating an odds ratio to 1.7, the prevalence of infection was estimated to be 35% and 55% in fertile and infertile women, respectively (15). We set the statistical significance level at 0.05 and test power at 80% by comparative study. We determined the sample size 230 for each group of participants. Finally, 234 infertile and 224 fertile women could complete the study.

The first group included infertile women in their childbearing age (18 to 49 years). Infertility defined as not being able to achieve pregnancy despite trying at least one year. Those with male factor infertility and antibiotic therapy within 30 days before the assessment were excluded from the study. The fertile group included women in third trimester of pregnancy admitted to delivery room. In this group the exclusion criteria were history of infertility, presence of genital tuberculosis, and recent antibiotic therapy.

**PCR:** Urinary sediments were extracted after centrifugation at 5000 rpm for 20 min and frozen at  $-70^{\circ}\text{C}$  in the laboratory of Children's Medical Center affiliated to Tehran University of Medical Sciences and they were subsequently transported to Avicenna Research Institute for PCR.

DNA was extracted from the pellets as described

by Sambrook and Russel and stored at  $-20^{\circ}\text{C}$  (16).

Amplification was performed by PCR method. The primer sequences for the amplification of *C. trachomatis* were as follow: DNA semi-nested or S: 5'-CTA-CGC-GTT-TGT-ACT-CCG-TCA-CA G-3', anti-semi-nested or AS: 5'-AAC-TTA-TCC-TCA-GAA-GTT-TAT-GCA-CTA -3'. PCR was performed in 25  $\mu\text{l}$  volume of a master mix containing 1 $\times$ PCR buffer (10 *Mm* Tris-HCl, pH=8.3, 50 *mM* KCl), 6 *mM* MgCl<sub>2</sub>, 0.2  $\mu\text{M}$  of each primers, 0.6 *mM* of each dNTPs, 1.0 *U* Taq polymerase, and 5  $\mu\text{l}$  of the template DNA. Initial denaturizing condition of this round was done at 94°C for 5 *min*, 37 cycles of 30 *s* at 94°C, 30 *s* at 60°C, 30 *s* at 72°C and a final extension at 72°C for 5 *min*. The PCR product of 206 *bp* was electrophoresed and visualized on a 1.5% ethidium bromide stained agarose gel (16).

PCR was performed on positive samples for 45 cycles, and semi-nested PCR on one of the negative samples to confirm the PCR in each series (n=8). The second-round mixture contained the same components. Two  $\mu\text{l}$  of the first round PCR product was used as the template DNA for the second-round amplification by using AS2: 5'-GGT-AAT-AAT-TTG-CTG-GAT-GGC-3' primers. The second-round amplification condition being the same as first-round. The PCR products were loaded on a 1.5% gel stained with ethidium bor-mide for electrophoresis.

**ELISA:** *C. trachomatis*-specific IgG, and IgM were determined in serum using Sero CP-IgG, -IgM protein ELISA; (EUROIMMUN, Germany), according to the manufacturer's instruction. Serum was designated as positive if the cut-off index was greater than 1.1. ELISA was done in Avicenna Research Institute.

**Statistical Analysis:** Diagnosis finding (IgG, IgM and PCR) were compared between study groups using the chi-squared test. Moreover, chi-squared was also used to compare *C. trachomatis* infection between the cause and type of infertility test. Logistic regression was used to show association between *C. trachomatis* infection and infertility. For this analysis, positive results of each diagnostic test (IgG, IgM or PCR) were separately used as dependent variables. Sensitivity, specificity, and positive and negative predictive values of ELISA tests (IgG and IgM) were also determined. The analyses were done by SPSS 13 (SPSS Inc, Chi-

cago IL, USA). A p-value less than 0.05 was considered as statistically significant.

### Results

In this study, we recruited 234 infertile and 223 fertile women. The mean age of the infertile and the fertile groups were 29.85 $\pm$ 6.26 and 26.85 $\pm$ 5.84 years, respectively. Infertility duration of the infertile women was 6.33 $\pm$ 4.70 years and the mean gravidity of pregnant women was 1.85 $\pm$ 1.08. The percentage of fertile women, with higher education (57.4%) was greater than the infertile group (8.37%).

Ovarian (101, 43.2%) and tubal (44, 18.8%) causes of infertility were more frequent than other causes of infertility. In 159 (67.9 %) women, the type of infertility was primary. In the pregnant group, 42 (18.8%) women had a previous history of abortion and 94 (42.4%) had pervious parities.

IgG about 21 (9.0%) infertile and 11 (5.0%) fertile participants were positive for IgG (p=0.093). IgM was positive in 2 (0.9%) women with infertility and 4 (1.8%) pregnant women (p=0.375) while PCR was positive for *C. trachomatis* infection in 29 (12.4%) women with infertility and 19 (8.5%) pregnant women (p=0.440). None of these differences were statistically significant.

In all the three diagnostic tests, *C. trachomatis* infection was not statistically significant between primary and secondary infertility. IgG antibody for *C. trachomatis* infection showed no statistically significant differences between causes of infertility (p=0.340). However, PCR showed higher rates of infection among infertile women with ovarian etiology (25.6%, p=0.002) than other etiologies (Table 1).

After adjusting for possible confounding factors in logistic regression analysis *C. trachomatis* positivity was not statistically significant in infertile against fertile women. The odds ratio was 1.499 (0.611 to 3.679) for IgG, 0.440 (0.064 to 3.013) for IgM and 1.254 (0.613 to 2.562) for PCR. Sen-

**Table 1.** Positive results of tests based on causes of infertility (n=234)

	PCR	IgG	IgM
<b>Uterus</b>	1 (4.3%)	3 (12.5%)	0
<b>Tubal</b>	2 (4.9%)	4 (9.7%)	1 (2.3%)
<b>Ovarian</b>	22 (25.6%)	4 (4.1%)	1 (1.0%)
<b>Other</b>	4 (7.0%)	9 (13.8%)	0
<b>Total</b>	29 (12.4%)	20 (8.7%)	2 (9.0%)

sitivity, specificity, positive and negative predictive values, and accuracy for IgG results were 6.3%, 92.5%, 10.7%, 87.3%, and 81.7%, respectively; for IgM they were 2.1%, 98.5%, 16.7%, 87.5%, and 86.4%, respectively.

### Discussion

Using different diagnostic methods, we found no significant differences between fertile and infertile women for *C. trachomatis* infection. Although this finding is compatible with some studies (10, 11), but some other studies have shown greater prevalence for *C. trachomatis* infection among infertile women and have mentioned it as an infertility risk factor (12). In case-control studies, group selection and control for confounding control can explain parts of these differences.

Prevalence of *C. trachomatis* in infertile women infection based on PCR and serologic detection of IgG and IgM respectively was 12.4%, 9.0% and 0.9%. In developing countries, this rate was reported to be 3.9% by PCR in Jordan (11) and 23.3% by direct immunofluorescence in Turkey (17). About 24% of infertile women had a rate lower than 10% in Western Europe. (18). These studies showed that about 10% to 30% of infertile woman had been infected by *C. trachomatis* Although a rate of about 12.4% in the present study is not indicative of a very high rate of infection, but it does not mean that the rate of *C. trachomatis* infection is low in Iran. In this study, we only evaluated patients in one of Tehran hospitals; therefore, the results cannot be generalized. Prevalence of *C. trachomatis* infection may be different in other areas in Iran, especially between rural and suburban areas.

These differences seem to be the result of differences in study settings, such as duration of study, socioeconomic status, sample size and diagnostic methods.

Prevalence of *C. trachomatis* may also vary in different groups of infertile women. There are some reports that *C. trachomatis* infection is more prevalent in tubal infertility than other types of infertility (15). Infection with *C. trachomatis* can cause adnexal adhesions and tubal obstruction (19) and it is the most common cause of tubal infertility (20). Therefore, it is rational to deduct that the infection is more prevalent in tubal infertility. Tubal assessment is recommended in infertile women with a positive result for *C. trachomatis* antibody (21). Our findings are compatible with the study done in northern Iran, which re-

ported the same prevalence for *C. trachomatis* infection in tubal and non-tubal etiologies for infertility (22).

*C. trachomatis* infection had the same prevalence in primary and secondary causes of infertility in the present study. This result is in line with study performed in Jordan (11); although there were also some reports indicating greater prevalence for the infection in secondary than primary infertility (12, 23). Having the same prevalence in different types of infertility, it is probable that *C. trachomatis* may induce infertility by other mechanisms than tubal damage, opening the door for further investigations.

An earlier study showed genital *C. trachomatis* is very common (24). Educational programs about sexually transmitted diseases is a known way of reducing the prevalence of these infections.

Enzyme-linked immunosorbent assay (ELISA) is the first generation of non-cultural tests to diagnose chlamydial infection. ELISA uses an enzyme-linked monoclonal or polyclonal antibody directed at the *C. trachomatis* lipopolysaccharide (25). In the presence of *C. trachomatis*, the antibody binds to LPS, and the linked enzyme induces a change in color that can be detected by a spectrophotometer. One benefit of ELISA is that specimens do not require refrigeration (25). In the present study ELISA showed low sensitivity but acceptable specificity. Other researchers have also reported low sensitivity (26) and adequate specificity for the test (27). Therefore, ELISA can be suggested as a screening test for detecting infection in patients with infertility. For those who have a positive result for *C. trachomatis* infection by this method, confirmatory tests are warranted. ELISA is also suggested for subgroups of patients with endocervical, urethral, or conjunctival specimens (25).

The sensitivity of PCR test for detecting *C. trachomatis* in endocervical samples has ranged from 51.9% to 96.8% (28–30). PCR has a reported sensitivity of 44.4% to 82.5% (29, 30) for detecting *C. trachomatis* in urine samples from women. Specificity PCR for *C. trachomatis* for samples from all reproductive organs has been reported to be 98.4% to 100% (30).

All in all, ELISA is recommended as a screening test for detecting *C. trachomatis* infection in individuals suspected of the infection but a confirmatory test needs to be done. Therefore, PCR can be used as the confirmatory test for diagnosis of *C. trachomatis*. Although nucleic acid amplification

tests (NAATs) need more time and are more expensive but their accuracy and non-invasiveness are noticeable. Additionally, Quantitative PCR is more sensitive than PCR because it detects lower numbers of microorganisms.

### Conclusion

It seems that *C. trachomatis* detection and treatment can be useful in infertile women improving their ART results. Due to the effects of *chlamydia* infection, tubal involvement and findings of this study, *Chlamydia* screening is highly suggested in infertile women before infertility management. Besides, prevention of maternal-fetal complications, indicates *C. trachomatis* screening during pregnancy.

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### Conflict of Interest

None of the authors had any conflict of interest.

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