

Non-detection of *Chlamydia trachomatis* infection by polymerase chain reaction in pregnant Iranian women

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Abstract

Chlamydia trachomatis is the most common cause of sexually transmitted infection. In 75% of women and 50% of men infection is asymptomatic. According to World Health Organization reports, the number of new genital infections with *Chlamydia trachomatis* reaches 100 million annually. The sensitivity and specificity of nucleic acid amplification tests are 95% and 99%, respectively. Urine samples can provide a non-invasive method of testing for the detection of *Chlamydia trachomatis* by polymerase chain reaction (PCR). The aim of this study was to evaluate the prevalence of *Chlamydia trachomatis* infection in pregnant women referred to a teaching hospital affiliated to Shiraz University of Medical Sciences. Urine samples were obtained from 210 pregnant women and investigated microscopically and macroscopically by urinalysis. Precipitants were also used for DNA extraction and PCR test for detecting *Chlamydia trachomatis*. Among 210 urine specimens from women aged 15-39 years, none were positive for *Chlamydia trachomatis* by PCR. In spite of the high sensitivity and specificity of PCR, and the elimination of inhibitory effects on PCR test, no pregnant woman was positive for *Chlamydia trachomatis*. Here, we suggest that a larger sample should be studied and other sensitive methods could also be used in the future.

Introduction

Chlamydiae are gram negative, non-motile and obligate intracellular parasites with a characteristic dimorphic growth cycle.¹ *Chlamydia trachomatis* (*C. trachomatis*) is the most common cause of sexually transmitted infection (STI).^{2,3} Urogenital chlamydial infec-

tions are caused by D-k serovars. Depending on the anatomical site of infection, *Chlamydia trachomatis* can cause urethritis, cervicitis, pelvic pain and proctitis. Untreated infection can cause severe complications, such as epididymitis, salpingitis, endometritis and pelvic inflammatory disease (PID) that can lead to infertility in men and women.^{4,5} *Chlamydia trachomatis* infection is asymptomatic in 75% of women and 50% of men.⁶ According to World Health Organization (WHO) reports, the number of new genital infections with *Chlamydia trachomatis* reaches 100 million annually.⁷ Neonatal conjunctivitis, pneumonia and nasopharyngeal infections can be caused by perinatal transmission during passage through the birth canal. Therefore, the Center for Disease Control (CDC) recommends universal screening of pregnant women.¹

Various risk factors, such as age, low socioeconomic status, multiple sexual partners, STI history and race are indicative of prevalence of infection. Studies report prevalence of infection to be 0-37% in pregnant women. Different diagnostic tests are available for screening *Chlamydia trachomatis*, such as nucleic acid amplification tests (NAATs) that have a sensitivity of 90-95% and specificity of 99%.^{3,8-10}

Polymerase chain reaction (PCR) is one of the NAATs which can be used. It offers a rapid, accurate and reliable method for detecting *Chlamydia trachomatis* in pregnant women.^{11,12} Urine and vaginal swabs provide samples which are used for screening *Chlamydia trachomatis* infection in subjects at risk. However, first voided urine (FVU) is a non-invasive sample and is convenient for collection, although it may have NAAT inhibitors.^{13,14} These are reported in 11.9% of pregnant women and in 5.2% of non-pregnant women.¹³

Studies on *C. trachomatis* infection among Iranian women showed variable prevalence rates (2.75-22%) according to different testing methods and samples.^{14,15} Considering the importance of asymptomatic chlamydial infections and the lack of recent data on the prevalence of *Chlamydia trachomatis* in Shiraz, Fars province, southwest of Iran, this study was conducted on urine samples of 210 pregnant women referred to Shooshtari Hospital, a teaching hospital affiliated to the Shiraz University of Medical Sciences.

Materials and Methods

Specimens

In this cross-sectional study from December 2010 to January 2011, 30-50 mL FVU samples were collected from 210 enrolled pregnant women referred to Shooshtari Hospital as a

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referral hospital for obstetrics and gynecological cases in Shiraz, Iran. Based on research questionnaires which were filled out by expert midwives, a scheme was drawn up according to age, education, place of residence, natural history of disease, history and types of infertility or miscarriage, premature delivery, ectopic pregnancy, contraception method, number of previous deliveries, and use of antibiotics. The urine specimens were collected in sterile plastic containers and rapidly shipped on dry ice to the Bacteriology and Virology Department of Shiraz Medical School.

Urinalysis

Considering the possible effects of urine inhibitory factors on PCR, such as β -HCG hormone and urine crystals, a complete urinalysis was carried out on all samples¹³ using a dipstick (Combi Labstick, Germany). Microscopic examination was also performed for each sample. Dipsticks were read according to the manufacturer's instructions. For microscopic examination, 10-15 mL fresh urine were centrifuged at 1500-3000 rpm for 5 min.¹⁶ A part of urine samples were kept at 4°C overnight to reduce PCR inhibitory factors.¹³

DNA extraction and polymerase chain reaction

Thirty milliliters of urine were centrifuged at 3000 rpm for 20 min. The precipitant was stored at -20°C until further processing. DNA

was extracted by a Bioneer DNA extraction kit (South Korea). PCR was performed in 30 μ L reactions (Cinnagene, Iran) containing 5 μ L extracted DNA plus 25 μ L of PCR mix with *omp1* gene coding region primers.¹⁷ PCR primer sequences were as follows: CT 90 UF: 3'-GGACATCTTGTCTGGCTTAACT-5' and CT 220 DR: 3'-GCGCTCAAGTAGACCGATATAGTA-5'. The reaction was performed by denaturation at 94°C for 5 min, followed by 35 cycles of 94°C for 1 min, 52°C for 1 min, and 72°C for 1 min, and a final extension for 10 min at 72°C.¹¹ A control without DNA was routinely included to detect possible contamination of the master mix components. *C. trachomatis* DNA confirmed previously by Gen-kam kit (Cat n. K014, Germany) was used as positive control. Urine samples were also tested with Gen-Kam PCR kits. PCR product (1492 bp for *omp1* gene and 315 bp for Genkam kit) was analyzed by electrophoresis in a 2% agarose gel with ethidium bromide staining.¹⁸ A 100 bp ladder (Vivantis, Malaysia) was used to identify the size of the PCR products.

Results

Among 210 urine specimens tested from women aged 15-39 years (25.62 ± 4.48), 48 (23%) were positive for bacteria, 38 (18%) were positive for crystals (calcium oxalate, amorphous urate, amorphous phosphate). Other factors were detected as follows: protein, 12 (6%) positive; WBC, 17(8%) positive; blood, 33 (16%) positive; keton, 4 (2%) positive; bilirubin, 2 (1%) positive; nitrite, 2 (1%) positive; sugar, 6 (3%) positive. All urine speci-

mens tested negative for *Chlamydia trachomatis* either by a Gen-Kam kit (Figure 1) or by our in-house PCR (Figure 2). According to such results no conclusions concerning the relationship of any of the above parameters to the infection could have been drawn.

Discussion

Asymptomatic infection with *Chlamydia trachomatis* can occur in 75% of women which makes diagnosis and treatment difficult. Lack or delay in diagnosis and proper treatment causes serious consequences, such as PID, infertility and ectopic pregnancy. Therefore, screening for *Chlamydia trachomatis* infection is one of the most important hygiene preferences for pregnant women. Infection can be detected by NAATs, such as PCR. Urine provides a reliable, effective and non-invasive sample for detecting *Chlamydia trachomatis*.¹⁹

We tested 210 urine samples from pregnant women. No sample was positive for *Chlamydia trachomatis* either using our in-house PCR or a Gen-kam PCR kit.

Considering the high sensitivity and specificity of the PCR tests used, and also the complete urinalysis for the detection and elimination of inhibitory factors on PCR tests, our results are consistent with some other reports. In a study conducted by Moussavi *et al.* in Tehran, Iran, on cervical discharge from 400 pregnant women exploiting the direct fluorescent antibody test (prevalence of *Chlamydia trachomatis* was reported to be 2.75%.¹⁴ Khazardoost *et al.* studied 1,114 pregnant women by ELISA and reported that prevalence

of *Chlamydia trachomatis* was 2.9%.²⁰ In another study in Australia on urine samples obtained from 1,175 pregnant women using two methods of PCR and ligase chain reaction (LCR), the prevalence of *Chlamydia trachomatis* was reported to be 2.8%.²¹ In a study on urine samples of 987 pregnant women by Chen *et al.* using PCR, the prevalence of *Chlamydia trachomatis* was reported to be 3.2%.²² In an extensive study of urine samples of 4,055 pregnant women using PCR carried out between 2003 and 2005 in the Netherlands, the prevalence of *Chlamydia trachomatis* infection was 3.9%.¹⁰ In a survey conducted on urine samples of 160 sexually active women in India, the prevalence of *Chlamydia trachomatis* in symptomatic women was 11.11%, while in asymptomatic subjects it was to be 7.14% (average 9.3%).¹¹ In a study carried out on urine samples of 978 Swedish women using PCR, the prevalence of chlamydial infection was 6%.²³ In a research performed by, Bozicevic *et al.*, in Croatia using more sensitive real-time PCR, the prevalence of *Chlamydia trachomatis* in 1,005 sexually active male and female subjects were reported to be to be 7.3% and 5.3%, respectively. In that method, two separate gene sequences (*omp1* and plasmid) were detected by PCR.²⁴ In a survey on endocervical swab using PCR in Singapore, the prevalence of *Chlamydia trachomatis* was reported to be 8%.²⁵

In a study of urine samples of 1,052 women using PCR by Tabriz *et al.* in Tehran, the prevalence of *Chlamydia trachomatis* was 12.6%. When positive samples were re-tested by single displacement amplification (SDA) the prevalence rate fell to 6.4%.²⁶ In this study, like the Gen-kam kit used in our study, plasmid

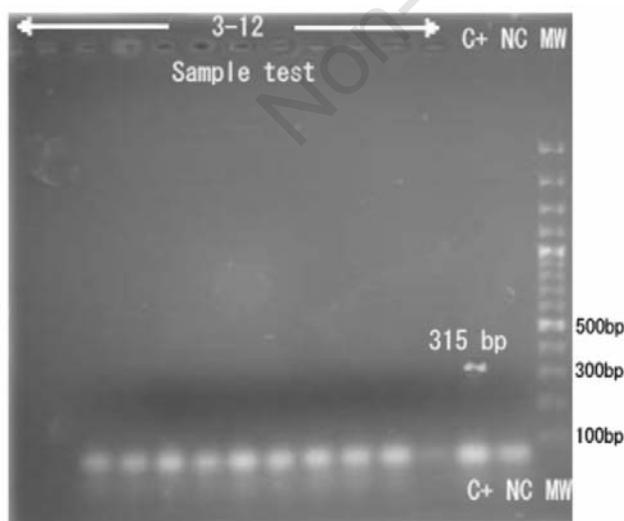


Figure 1. Image of the ethidium bromide stained gel of 315 bp band in urine polymerase chain reaction products. MW, 100 bp molecular weight ladder. Lane 1, negative control, Lane 2, positive control, Lanes, 3-12 urine samples.

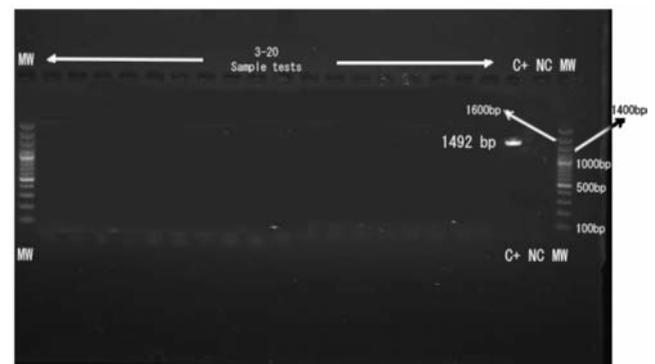


Figure 2. Image of the ethidium bromide stained gel of the 1492 bp *omp1* gene band in urine polymerase chain reaction products. MW, 100 bp molecular weight ladder. Lane 1, negative control, Lane 2, positive control, Lanes, 3-20 urine samples

DNA was used for detection. As 10 copies of the plasmid exist in elementary bodies of *Chlamydia trachomatis*, detection of infection is more likely. However, it has been reported that these plasmids are sometimes cryptic and may not often be detected.² Another difference is that 39% of participants had symptoms of infection, including vaginal discharge.

In a serological study by Bakhtiari *et al.* using ELISA on blood samples of women referred to a clinic, the prevalence of *Chlamydia trachomatis* was 11.6%; however, in Mazandaran this was reported to be 2.75%.²⁷ That research is different from our study in sample size and method, making it not as sensitive and specific as ours.²⁸

In two studies performed in Iran: one in Ahwaz by Samarbaf Zadeh *et al.* on 202 vaginal samples using PCR, and the other by Hashemi *et al.* in Tehran on endocervix samples of 123 married women with cervicitis using by PCR and enzyme immunoassay (EIA) the prevalence of *Chlamydia trachomatis* was 16.2% and 17%, respectively.^{29,30}

In another study conducted on urine samples of 140 married women in Tehran which also used the omp 1 gene, the prevalence of *Chlamydia trachomatis* was reported to be 22.1%. However, this study differed from ours in two respects: participants were not pregnant and nested PCR was used.¹⁵

In the only relevant research in the Fars province of Iran, carried out by Hadi *et al.*, on 402 Pap smear samples of women attending a gynecological clinic using an immunofluorescence method, the prevalence of *Chlamydia trachomatis* was 8%.³¹ Here, 219 participants had symptoms such as vaginal discharge and dysuria and the method used was less sensitive and less specific than that used in our study.³¹

One way in which our study differed was that complete urinalysis was carried out on all samples, among which 38 samples had urinary crystals, including calcium oxalate, amorphous ureate and amorphous phosphate. With regards to the inhibitory effects of urinary crystals and also β -HCG hormone on NAATs this test could be useful. Keeping urine samples at 4°C overnight will reduce the inhibitory effect to a minimum.¹³

In a study carried out on urine samples of pregnant and non-pregnant women, the inhibitory effect of various factors on NAATs was reported to be 11.9% in pregnant women and 5.2% in non-pregnant women.¹³

Conclusions

All urine samples of the 210 pregnant women in our study tested were negative for

Chlamydia trachomatis by PCR, using sensitive in-house PCR and Gen-Kam kit. It should be noted that moral, social and religious factors in our society could have resulted in this low chlamydial infection rate among the normal population in contrast to in subjects who have multiple sexual partners. However, a larger sample size in future studies will provide more precise data.

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