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Detection of *Chlamydia trachomatis* from Urine Specimens by PCR in Women with Cervicitis

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Abstract

Chlamydia trachomatis is the most common agent of urogenital infections in both men and women. Diagnosis of chlamydial infections is based on isolation of bacteria in tissue culture media that requires at least 48 to 72h. Polymerase chain reaction (PCR) is a sensitive and specific method for detection of small quantity of bacterial DNA in clinical samples. The first goal of this study was to perform a PCR testing for detecting of *C. trachomatis* from urine samples and after that to identify the frequency of *C. trachomatis* among cervicitis women and at the end, to identify the potential risk factors for chlamydial genital infection. From August to October 2002, a total of 122 consecutive women with cervicitis who attended Obstetric & Gynecology Clinic of Shoosh, Tehran-Iran were involved into the study. After DNA extraction from urine specimens, PCR tests were performed. *C. trachomatis* genome was detected in 14 of 94 (14/9%) urine specimens. The highest *C. trachomatis* cervical infection frequency was found in women with 28 to 38 years old group, elementary education level group, and in users IUD for contraception. The results of this study indicate that PCR technique is a useful method for detecting *C. trachomatis* in urine.

Keywords: Chlamydia trachomatis*, Cervicitis, Iran*

Introduction

Chlamidia trachomatis is one of the most common causes of treatable sexually transmitted diseases in both men and women (1-3). Worldwide, there is an estimated annual incidence of 50 million cases of chlamydial infections (4). *C. trachomatis* species is divided into fifteen serovars defined by polyvalent antisera and monoclonal antibodies. Serovars A, B, Ba and C are the aetiological agents of trachoma; servers D-K are associated with oculogenital infections, while serovars L1, L2 and L3 cause lymphogranuloma venereum (5).

Urogenital infections with *C. trachomatis* in women have a clinical course varying from asymptomatic infections to ascending infections leading to pelvic inflammatory disease associated with late ectopic pregnancy and tubal infertile (6). *C. trachomatis* can cause many human diseases, including ocular trachoma, urethritis, epididymitis, cervicitis, salpingitis and lymphogranuloma (7). Cervicitis is the main genital infection caused by *Chlamydia* in females (8). In an earlier investigation, *C. tra-*

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chomatis cervical infection was also associated with preterm premature rupture of the membranes, preterm labor and low birth weight (9).
Asymptomatically infected women may be at risk of serious reproductive squeal; therefore, screening for genital chlamydial infection is a high public health priority (10). The increasing prevalence of chlamydial disease has generated much interest in development of sensitive, specific, and rapid techniques for diagnosis of chlamydial infections (11). Diagnosis of *C. trachomatis* infection is frequently based on bacterial isolation in tissue culture media. This method requires careful specimen collection and stringent transport condition and requires at least 48 to 72 h to perform (4).
Recently, the PCR method has been used to detect *C. trachomatis* infections. PCR has proved to be more sensitive and specific than the conventional microbiological assays (2-4, 10-14).
Nucleic acid amplification techniques such as PCR involve exponential amplification of well-defined DNA targets, resulting in enhanced sensitivity of detection compared with the sensitivities of other nonculture methods (10). The PCR is a new, in vitro DNA amplification technique that allows a virtual exponential amplification of a well-defined DNA molecule during several cycles, the advantage of DNA amplification by PCR is that it does not require live organisms. In addition, PCR appears to be an optimal tool for enhancement of the sensitivity of detection (11).The previous studies shows that PCR has a sensitivity of 97% to 100% and a specificity of 98% for detecting *C. trachomatis*, while culture has a sensitivity and specificity of 85% and 100%, respectively (11- 15).
The objective of this study was to perform a PCR testing for detecting *C. trachomatis* in urine samples determine the frequency of *C. trachomatis* among cervicitis women identify some potential risk factors for chlamydial genital infection.

Materials and Methods

Patients From August to October 2002, a total of 122 consecutive women with cervicitis who attended Obstruct & Gynecology Clinic of Shoosh, Tehran-Iran, involved into the study. After examination by physicians, informed consent was obtained from all patients, and a questionnaire regarding, age, level of education, job, marital status, history of genital infections, history of aborting, contraceptive methods, history of genital surgery and symptoms was completed. None of the patients had received antibiotic treatment 4 weeks prior to the visit and were eligible if they had not urinated during the previous 2 hr. Ten to 15 ml of urine from each patient was collected into sterile cups and stored immediately at 4° C until processed. The urine specimens were centrifuged at (12,000× g) for 20 min and the supernatant was discarded and the pellet was frozen at- 20°C, until the PCR was performed.

DNA extraction from specimens The pellet was suspended in 500 µl of sodium dodecyl sulfate (SDS) lysis buffer [50 mM Tris-EDTA (PH 7.5), 1% SDS, 0.1 mg of proteinase k per ml]. The suspension was incubated at 55°C for 4 hr and then was boiled for 20 min. The lysate was extracted with an equal volume of phenol and then with equal volume of chloroform. DNA was precipitate in an equal volume of isopropanol, washed on time with 70% ethanol, dried, and resuspended in 50 µl of double-distilled water (7).

Polymerase Chain Reaction *C. trachomatis* is an obligate intracellular bacterium (16). The outer membrane proteins of obligate intracellular bacteria play a direct role in the process of adaptation by facilitating interactions between the bacterial cell and its host cell (7). Thus, MOMP has been implicated in the mechanisms of attachment, infection and pathogenesis (7).
The primers;
CT1:CCT/GTG/GGG/AAT/CCT/GCT/GCT/GAA
and
CT4:GTC/GAA/AAC/AAA/GTCATCCAGTA/GT
A were derived from highly conserved regions of the published DNA sequences for the MOMP of *C. trachomatis* serovars (9, 16, 17).

(Accession number: gi/ 532121/ gb/ L35606.1/ CHTOMP1A). All serovars produced the same intensity 144-bp fragment (16).

In brief, 2 µl of DNA extracts was processed in a 30 µl reaction volume containing PCR buffer (10 mM Tris [pH 9.0], 50 mM KCl, 0.01% gelatin), 200 µM deoxynucleoside triphosphates, 2.5 mM MgCl2, 0.5 µM each primer, and 1 U of Taq polymerase. Amplifications were carried out in a mastercycler (Eppendrof, Germany).The first cycle, consisting of a 5-min denaturation at 94° C, was followed by 35 cycles each of 30 s at 94° C, 45 s at 56° C, and 1 min, at 72° C, with a final extension for 10 min at 72° C. The PCR products were visualized in 2% agarose gels containing 0.5 µg of ethidium bromide/ml. Then we confirmed all the chlamydial positive specimens by using primers against plasmid of *C. trachomatis* that were designed by using DNASIS software. Primers BP1: AAC/ CGT/ TTT/ TAA/ TAG/ TGGCA and BP2: TTC/ TGG/ CCA/ AGA/ ATT/ ATCC (18) which amplify a 377-bp fragment of chlamydial cryptic plasmid (7.5-kb). The *C. trachomatis* cryptic plasmid is used as target DNA for amplification because there are 10 copies per elementary body; sensitivity is strongly increased (11). With advances in DNA technology, laboratory methods for the amplification and detection of the multicopy plasmid DNA present in all *C. trachomatis* serovars have been introduced for the diagnosis of *C. trachomatis* infection (2).

Results

DNA was extracted only in 94 specimens from all 122 collected urine specimens, and 28 specimens were lost. Fourteen of 94 specimens (15%) were positive by PCR using specific primers for MOMP (CT1 & CT2) (Fig. 1) and cryptic plasmid (BP1 & BP2) (Fig. 2). The results of PCR by MOMP primers (CT1 & CT2) and cryptic plasmid primers (BP1 & BP2) were the same.

In our study patients' age ranged from 17 to 62 years and all of the *Chlamydia* positive patients were housekeepers. The age distribution of the patients is shown in Table 1 and the level of education in Table 2. All positive cases were married, and did not ever used condom. Five (36%) of them used IUD, 3 (21%) used oral contraceptives, for 2 patients, tubectomy was done and 4 (29%) did not use any contraceptive methods.

Eleven of 14 (79%) *C. trachomatis* positive patients had a history of genital infections, 5 (36%) had a history of abortion, and 8 (57%) had a genital surgery in the past. The clinical symptoms in *C. trachomatis* positive patients are shown in Table 3.

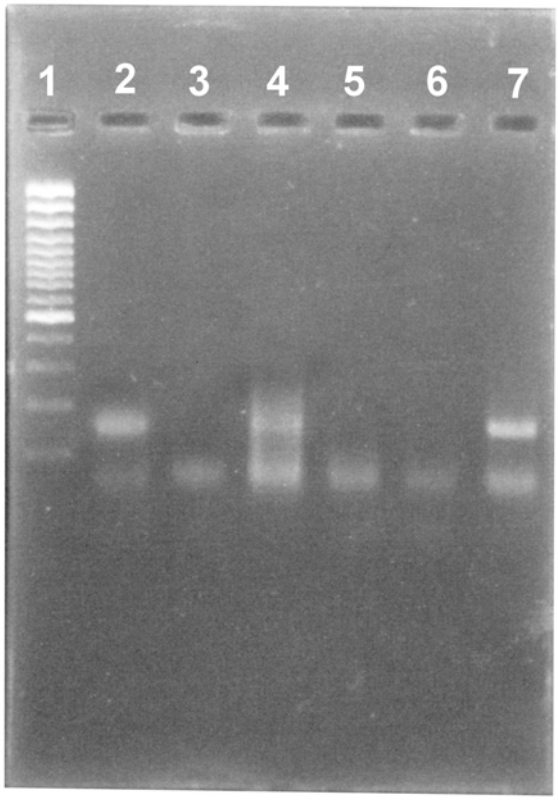

Fig. 1: Detection of *C. trachomatis* from cervical specimens by PCR (MOMP primers). Lane 1, DNA size markers; lane 2, positive control; lane 3, negative control; lane 4 and 7 positive patients for *C. trachomatis* and lane 5 and 6 negative patients for *C. trachomatis*.

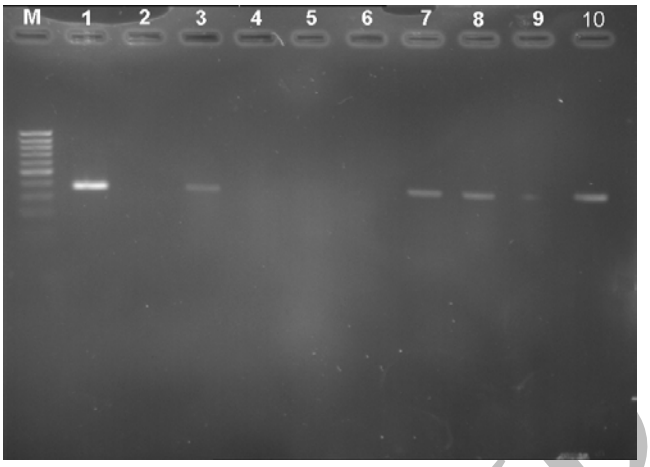

Fig. 2: Detection of *C. trachomatis* from cervical specimens by PCR (plasmid primers). Lane M, DNA size markers; lane 1, positive control; lane 2, negative control; lanes 3, 7, 8 and 10 positive patients for *C. trachomatis*.

Table 1: Frequency of *C. trachomatis* among cervicitis women according to age

Age (y)	PCR positives No.	PCR positives %
17-27	4	28.57
28-38	6	42.8
39-49	4	35.71
50-60	-	-
>60	-	-
Total	14	

Table 2: Frequency of *C. trachomatis* among cervicitis women according to level of education

Level of education	PCR positives No.	PCR positives %
None	1	7.14
Elementary	6	42.85
Intermediate	5	35.71
High school	2	14.28
University	-	-
Total	14	

Table 3: Frequency of symptoms in cervicitis women

Symptoms	PCR positives No.	PCR positives %
Fever	5	35.71
Mucopurulent cervicitis	13	92.85
Painful intercourse	7	50
Painful urination	5	35.71
Pain in lower abdomen	5	35.71
Itching	9	64
Abnormal menstrual periods	4	28.5

Discussion

Collection of samples for *C. trachomatis* diagnostics has often caused discomfort and sometimes embarrassment for the female patients. Urine is usually a better sample compared with swab. Collecting a urine sample is better for patients (12). It is simpler to obtain a proper urine sample and does not require medical personnel to perform a vaginal speculum examination. It can be argued that omitting examination in the clinic may cause other diseases, like genital warts, to be missed (12).

Culture was earlier considered the gold standard, but PCR studies suggest that the sensitivity of the culture even in expert lapidaries is as low as 75% to 85%. (2). It is universally accepted that culture can no longer serve as a reference method in the evaluation of diagnostic tests for *C. trachomatis* (2). Another advantage of urine samples tested with amplification assay is that pooling of samples will not compromise the sensitivity but reduces cost. Several studies have shown that using DNA amplification methods on urine samples for detection of *Chlamydia* is cost-effective (19). Jorma Paavonen et al., advocated the use of a PCR test for urine (19). PCR is probably the most sensitive *C. trachomatis* nonculture test now available (20).

The use of nucleic acid amplification methods

such as PCR has significantly improved our ability to diagnose genital *C. trachomatis* infections. These methods also allows for the use of noninvasive urine specimens for testing which are more acceptable to patients (21).

The PCR has recently been reported as a method for detecting the major outer protein (MOMP) gene from the 15 *C. trachomatis* serovars (16).

The sequence analyses of MOMP genes show that genes have both constant and variable domains (5). All serovars of *C. trachomatis* MOMPs have several regions of highly conserved nucleotide sequence, which were used for primer selection (16).

This assay gives more true-positive results than cell culture. Therefore, it is good alternative to culture and should be readily accepted for routine use (11). The most attractive aspects of DNA amplification methods are their excellent sensitivities and good performance with urine samples. To prevent loss of sensitivity of the PCR assay, all of the samples were subjected to a standard phenol-chloroform extraction and ethanol precipitation. DNA extraction was largely successful and the samples were mostly free of potential inhibitors. Highest *C. trachomatis* cervical infection frequency was found in women with 28 to 38 years old group (Table 1), elementary education level group (Table 2), and IUD users for contraception. We found the common sign in our patients is mucopurulent (Table 3). Infected women at their sexual active age form an important reservoir of transmission and untreated women are possibly at risk of developing sequels. The results of this study show using condom has a protective effect against *C. trachomatis* infection and using IUD increases risk of chlamydial cervicitis.

There are several studies that determined the frequency of *C. trachomatis* in women with cervicitis. A study in Papua New Guinea determined the prevalence of 17% by using PCR and direct immunofluorescence assays (22). In Australia, the prevalence ranges from 2.5 to 14%, with the highest rate among patients attending sexually transmitted disease clinics (3). Another study in Senegal shows that prevalence of *C. trachomatis* in Dakar commercial sex workers is 29% (23). Although it is higher than our result (15%), the high-risk behavior of that study population can explain such a high prevalence of *C. trachomatis* genital infection.

There are few studies in Iran. Darugar et al., isolated *C. trachomatis* from cervix of in this regard 6.9% of infected women in 1978 (24). Another study determined the prevalence of *C. trachomatis* in Tehran as 22% and in Bandar Abbas as 10% (24). Also another study showed the prevalence of *C. trachomatis* in infected women in Shiraz as 6.5% by cell culture and microimmunoflorsence techniques (25).

The reported frequency in our study is unexpectedly high, particularly for populations which were considered low risk. The convenience of using urine samples for testing female patients is a major step in the detection and control of this STD. Urine screening is important because it represents a noninvasive method for detection of *C. trachomatis*.

In summary, nucleic acid amplification methods, such as the PCR, are significantly more sensitive and should therefore be used in preference to other tests for the detection of genital *C. trachomatis* infection.

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