Original Article

Determination of Asymptomatic *Chlamydia Trachomatis* Infections by Omp1 Gene Based -PCR

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Abstract

Received: 25/Nov/2007, Accepted: 6/Feb/2008

Objective: The objective of this research was to determine the prevalence of genital *C. trachomatis* infection in asymptomatic women by using highly sensitive nested-polymerase chain reaction (PCR) in urine sample.

Materials and Methods: One hundred-forty asymptomatic women were randomly selected from those who attended gynecology out patient department of Hazraat e Rasool Hospital in Tehran. First catch urine specimen were collected from all the participants. DNA extraction was performed by means of High Pure PCR Template Preparation Kit (HPPTP) according to the manufacture's instructions. Extracted DNA was tested by omp1 gene based nested-PCR, using sets of primers to amplify *C. trachomatis* omp1 gene. Visualization of a 1027 bp fragment from omp1 gene in agarose gel electrophoresis was considered as a positive result.

Results: In total, 140 urines were tested for determination of *C. trachomatis* infection. *C. trachomatis* omp-1 was detected in 22.1% of cases (31/140). The overall prevalence rates of *C. trachomatis* in the urine sample as determined by omp1 based nested-PCR were 4.3% in group I (age, <25 years), 12.1% in group II (age, 25-34 years), 5.0% in group III (age, 35-44 years) and 0.7% in group IV (>44 years). The highest prevalence of *C. trachomatis* infection (12.1%) was seen in women aged 25-34 years. This finding was not statistically significant (p=0.710). Also, there was not relation between *C. trachomatis* infection and some probable risk factors such as young age (<25 years), STD history and missing use of barrier contraceptive in this study.

Conclusion: The prevalence of *C.trachomatis* infection in the women not seeking health care warrants more comprehensive study using high sensitive omp1 based nested- PCR to identify and treat a large number of infected women in Iran.

Keywords: *Chlamydia Trachomatis*, Asymptomatic Infection, omp1 Gene, Nested-PCR, Urine Sample

Yakhteh Medical Journal, Vol 10, No 1, Spring 2008, Pages: 41-46

Introduction

Worldwide, *Chlamydia Trachomatis* is one of the most common causes of sexually transmitted disease (STD), with an estimated 90 million new cases per year (1). In women, more than 70% of urogenital *C. trachomatis* infections are asymptomatic. These asymptomatic women are at greatest risk of developing serious complications such as pelvic inflammatory disease (PID), ectopic pregnancy, infertility, chronic pelvic pain, pregnancy outcome and increased susceptibility to HIV (2-6).

Considering the high rate of asymptomatic chlamydial infection, identification and treatment of these women is important in prevention of the subsequent complication. In many areas, diagnosis of *C. trachomatis* genital infection is only performed in selected

populations, often based on the presence of clinical symptoms and there are few studies in asymptomatic population (7-9).

Within the last decade, tests that are based on nucleic acid amplification such as PCR have become available to detect *C. trachomatis* infection with high sensitivity and specifity. They are suitable especially in asymptomatic population since these infections are often associated with the low copy number of bacteria (6, 10-12).

In order to study the epidemiology of C. trachomatis infections, PCR is performed based on omp1 (outer membrane protein) gene as a target. Omp1 based PCR was done successfully with both genital and urine samples and showed no cross-reaction with any bacteria or other false-positive reactions. A positive omp1 PCR is the criteria for a "true positive" result as well as a positive cell culture. Specifity and sensitivity of higher than 99.5% and 90% respectively are considered for omp1 PCR (10, 13-15).

The omp1 based nested-PCR not only detects the infection, but also is used to genotype various *C. trachomatis* serovars by PCR-RFLP (restriction fragment length polymorphism) and sequencing of the amplified omp1 gene. Therefore the omp1 gene detection provides a valuable and sensitive means for molecular epidemiological analysis (16, 17).

The omp1 gene encodes the major outer membrane protein (MOMP), the main surface protein component and immunodominant antigen of *C. trachomatis*. It has been suggested that MOMP also functions as a chlamydial cytoadhesin. Functional MOMP probably serves as a porin, regulating the chlamydial developmental cycle by the passage of small molecules through the outer membrane (18, 19).

The objective of this research was to determine the prevalence of urogenital *C. trachomatis* infection in asymptomatic women by using highly sensitive nested-PCR based on omp1 gene in urine sample.

Materials and Methods

Patients and specimens collection

A total of 140 asymptomatic women were randomly selected from those who attended gynecology out patient department of Hazraat e Rasool Hospital in the city of Tehran, Iran from March to December 2006.

Asymptomatic women were defined as those who did not have any symptoms suggestive of urogenital tract infection including: abnormal vaginal discharge, spotting, post-coital bleeding, dysuria, lower abdominal pain, dysmenorrhea and dispareunia and contacted a physician for a variety of other reasons like contraceptive advice, retarded menstruation or routine gynecologic care (9).

All the women who were not married,, not sexually active, ages <15 or >50 , or being pregnant were excluded from the study. Questionnaires were filled to collect demographic and sexual history details. Clinical backgrounds including age, use of condom and STD history were noted. All the women who did not have urination during the past two hours, were asked to collect 20-30 ml of the first void urine (first part of urine) in a sterile plastic bottle,

Sample preparation for PCR

The urine samples were kept over night at 4°C to decrease the probable inhibitory elements, and then centrifuged at 3500 g for 20 min. The pellets were washed twice with PBS (phosphate buffered saline) and then resuspended in 200µl PBS and frozen at -20º C before testing. DNA extraction was performed by means of High Pure PCR Template Preparation (HPPTP) Kit (Roche Diagnostic GmbH, Germany) according to the manufacture's instructions.

Nested-PCR of omp1 gene

Ten µl of the prepared DNA template was used in the 50 µl primary PCR reaction using first PCR primers(CT-1 and CT-2) with the 3′ nucleotides at positions -43 and 1119 in the omp-1 gene sequence (Table 1). The thermocycler profile was: DNA denaturation at 94ºC for 5 minutes followed by 30 cycles of amplification. Each cycle consisted of a denaturation step at 94 º C for 30", an annealing step at 58 º C for 30" and a chain elongation step at 72ºC for 1 min. The final elongation step was extended for another 5 min. Two µl of the primary PCR product were used for secondary PCR which was prepared and run using the same reagents and conditions as the primary PCR, except for primers with the 3′ nucleotides at position 18 and 1084 respectively (CT-3 and CT-4) (Table 1) and annealing temperature that was 55ºC. Ten µl of amplicon was electrophoresed using 1.5% agarose gel in TE buffer and visualized by transluminator.

The DNA Ladder, Low Range (Fermentas) was included in each electrophoresis and also, positive and negative controls were included in each run of test.

Table 1: Primers used for nested- PCR of omp1 gene

Primer	Sequence (5′-3′)	Reference
CT-1	ATGAAAAAACTCTTGAAATCGGTA	Stephens et al 1986
CT-2	TTAGAAGCGGAATTGTGCATTTAC	Stephens et al 1986
CT-3 (PCTM3)	TCCTTGCAAGCTCTGCCTGTGGGGTCCT	Lan et al 1993
CT-4 (NR1)	CCGCAAGATTTTCTAGATT	Lan et al 1993

Statistical analysis

Data were analyzed with SPSS statistical software version 11.5 for Windows. Equality of mean age in infected and non-infected women was calculated using t test. The results of the nested-PCR, and each risk factor and also age groups were assessed using χ^2 (Chi square) test. Odds ratios (OR) with 95% confidence interval (CI) were calculated. P value of less than 0.05 was considered to indicate statistical significance.

Ethical approval

This study was approved by the ethical committee of the research council of Iran University of medical sciences.

Results

In this cross-sectional study the mean age of 140 participated asymptomatic women was 30.12 (SD=6.2) years. Among the enrolled women, 23.6% were under 25, 50.8% in 25-34 and 24.2% in 35-44 age groups and 1.4% over 45 years of age (Table 2).

Figure 1 shows the agarose electrophoresis of amplified omp1 gene by two pairs of primers from urine samples. Visualization of a 1027 bp fragment in agarose gel electrophoresis was considered as a positive result for *C. trachomatis* infection because of specifity higher than 99.5%. Thirty-one of these women (22.1%) were positive for *C. trachomatis* infection using omp1 based nested-PCR. The mean age of *C. trachomatis* infected and non-infected women was 30.9 (SD=6.7) and 29.8 (SD=6.1) respectively and *C. trachomatis* infection was not related to the age (p value=0.40, Mean Difference=1.077, CI 95%, -1.447-3.602). Table 2 shows the overall prevalence rates of *C. trachomatis* infection were 4.3% in group I (age, <25 years), 12.1% in group II (age, 25-34 years), 5.0% in group III (age, 35-44 years) and 0.7% in group IV (>44 years). The highest prevalence of *C. trachomatis* infection (12.1%) was seen in women aged 25-34 years. This finding was not statistically significant (p=0.710).

Table 2: Prevalence of C. trachomatis infection in asymptomatic women according to age group

Age groups (yr)	Positive No (%)	Negative No (%)	Total: No of patients (%)
I (<25)	6 (4.3)	27 (19.3)	33(23.6)
II (25-34)	17 (12.1)	54 (38.7)	71(50.8)
III (35-44)	7 (5.0)	27 (19.2)	34(24.2)
IV (>44)	1 (0.7)	1 (0.7)	2(1.4)
Total	31(22.1)	109 (77.9)	140(100)

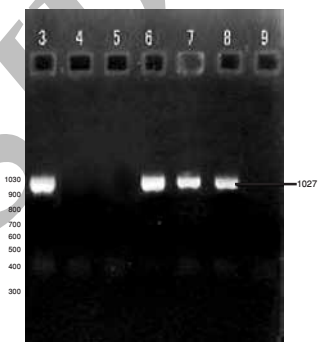

Fig 1: Agarose gel electrophoresis of amplified C. trachomatis omp1 gene from urine samples: Lane M, DNA Ladder (size marker), Low Range (Fermentas); Lanes 1, 3, 6, 7, the positive clinical specimens by nested-PCR showing the 1027 bp amplicon; Lanes 2, 4, 5, the negative clinical specimens; Lane 8, positive control of C. trachomatis showing the 1027 bp amplicon; lane 9, negative control (distilled water).

Table 3: Association of probable risk factors in C. trachomatis infection

Risk factors	PCR positive	PCR negative	OR (95%CI)	p-value
Age Under 25 years	6	27	0.729 (0.270-1.965)	0.531
25 years or over	25	82		
STD history Yes	16	52	1.169 (0.526-2.598)	0.701
No	15	57		
Barrier Contraceptive Yes	4	18	0.749 (0.234-2.402)	0.629
No	27	91		
Total	31	109		

Some probable risk factors for chlamydial infection such as young age (<25 years), previous STD history and barrier contraceptive use missing were investigated in this study. These factors were not related to *C. trachomatis* infection by means of χ^2 test. P -values and odds ratios were presented in table 3.

Discussion

In this study using the omp1 based nested-PCR with urine samples, showed that the prevalence of *C. trachomatis* infection was 22.1% in asymptomatic women.

The PCR method has proved to be more sensitive and specific than conventional microbiological assays for detection of *C. trachomatis*. Also with the introduction of a more appropriate combination of a primary PCR and a nested PCR with two set of primers for amplification of omp1 gene, the overall sensitivity was improved. So, omp1 based nested-PCR is a valuable tool for studying the prevalence of asymptomatic *C. trachomatis* infections, especially since these infections are often with low copy numbers of *C. trachomatis* (12-14).

This was the first study to use a highly sensitive omp1 based nested- PCR assay to examine the women not suspicious for *C. trachomatis* and thus allowed us to better estimate the real magnitude of the asymptomatic carriers of *C. trachomatis* infections in Iranian population.

Few studies conducted in clinical settings in Iran based on the presence of symptoms. They reported prevalence of 14.9% - 22.4% in symptomatic women by using plasmid based PCR (20-23). The prevalence of *C. trachomatis* in our asymptomatic population was expected to be lower than symptomatic women. It is likely that the high rate of *C. trachomatis* infection in this study (22.1%) may be due to the high sensitivity of omp1 based nested-PCR as well as sample processing and selection of the population from different clinics. Unlike some plasmid free strains of *C. trachomatis*, all strains of this bacterium possess omp1 gene (24). Therefore contrary to plasmid based techniques, in omp-1 amplification based techniques all strains of *C. trachomatis* are detected.

Studies in other countries such as France, UK, Slovenia and USA showed that the prevalence of *C. trachomatis* in general population, including asymptomatic women was 1.6-11.6% (3, 8, 15, 25-27). By finding a high rate of asymptomatic chlamydial infection in Iran, It is time to increase research efforts to identify and treat chlamydial infections in asymptomatic as well as symptomatic people, utilizing high sensitive omp1 based nested- PCR. Because of high sensitivity of omp1 gene for identification of *C. trachomatis*, by using this method, most of infected women would be identified.

Also some studies showed the correlation between chlamydial infection and young age (less than 25 years) as well as history of any sexually transmitted disease (STD) and barrier contraceptive (condom) use missing (2, 25, 28-30). This association with young age is largely attributable to the higher level of sexual activity among younger women. On the other hand it seems that the presence of one STI (sexually transmitted infection) increases the likelihood of concurrent infections and leads to contracting and transmitting *C. trachomatis* as well as other STIs. Therefore any history of STD can be considered as a risk factor for genital chlamydial infection. In our study, STD history and condom use missing were not related to *C. trachomatis* infection which is in agreement with Cameroonian study (31). As opposed to the results of studies conducted western countries, in this study high prevalence of *C. trachomatis* infection was in 25-34 age group. This difference may be due to beginning of sexual activity in young age before marriage in western countries and un-married status is considered as a risk factor in these countries. However due to cultural and social differences, sexual activity begins usually after marriage in Iranian population. So in comparing prevalence across different studies, one needs to take into account the background risk of included women and the testing technique employed, as this would influence the result of the study.

Conclusion

In conclusion, because our population was not made up of women seeking health care, by finding high prevalence of *C. trachomatis* in a normal population of women, more comprehensive study using omp1 based nested- PCR to identify and treat a large number of infected women in Iran is recommended. The identification and treatment of infected persons not only reduce the burden of disease in individuals

but also prevent the spread of *C. trachomatis* in society.

Acknowledgements

We thank all of the women who kindly participated in this study. Also, we would like to thank the medical personnel at the obstetrics and gynecology section and central laboratory of Hazraat e Rasool Hospital, microbiology and immunology departments of Iran University of medical sciences and cellular & molecular biology research center of Shaheed Beheshti University of medical sciences in Tehran, Iran. This research was financially supported by Research Council of Iran University of Medical Sciences.

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