

Frequency of *Chlamydia Trachomatis* in Symptomatic and Asymptomatic Non-pregnant Women in Golestan Province

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ABSTRACT

Background and Objective: *Chlamydia trachomatis* is one of the most common causes of genital infection in men and women. Genital chlamydial infections in women are clinically asymptomatic in 70-80% of the cases; therefore, the lack of timely diagnosis and treatment leads to complications such as infertility and ectopic pregnancy. The aim of this study was to evaluate the frequency of chlamydial infection in symptomatic and asymptomatic women in the Golestan province.

Methods: This cross-sectional study was conducted on 150 cervical swab samples obtained from 150 women referred to the clinic, after obtaining written consent and completion of questionnaires. The swab samples were transferred to laboratory in phosphate-buffered saline solution and DNA extraction was carried out using phenol-chloroform and boiling methods. The frequency of chlamydial infection was evaluated by PCR.

Results: None of the tested samples were found as *Chlamydia*-positive.

Conclusion: The findings require that some more extensive research with larger sample sizes and dispersed population be performed to determine the true prevalence. Considering the serious complications of chlamydial infections and its asymptomatic nature, a highly sensitive and specific method such as PCR should be used to detect Chlamydia. It is suggested that this method be used along with a complementary test to obtain the results that are more accurate. Furthermore, conducting simultaneous studies on other populations at risk will be very helpful in obtaining representable national data.

Keywords: Chlamydia Trachomatis, PCR, Vaginal Swab.

INTRODUCTION

Chlamydia trachomatis is a small coccoid, Gram-negative and immobile bacterium living as an obligate intracellular parasite of humans and animals (1). *C. trachomatis* is one of the most common causes of sexually transmitted diseases (STD) in men and women that are treatable (2).

According to the World Health Organization, approximately 92 million new cases of chlamydial infection occurs in the world, and 4-5 million of these new cases happen only in the United States (3).

Chlamydia causes nongonococcal urethritis and epididymitis in men, Reiter's syndrome or proctitis and conjunctivitis in men and women, and cervicitis, urethritis, endometritis, salpingitis and perihepatitis in women (4).

More than half of *C. trachomatis* infections are asymptomatic in both men and women. If not treated properly, they can lead to severe complications such as pelvic inflammatory disease, ectopic pregnancy and infertility in women (2).

The *C. trachomatis* infection in cervix may be transmitted to the baby during childbirth, which leads to pneumonia and conjunctivitis in newborns (4).

The prevalence of *C. trachomatis* infection has been estimated 6.5% to 25% in Iran and other countries (5). Moreover, the prevalence of asymptomatic infections in other countries has been reported between 1.6% and 19% depending on the population studied and the methods used. These individuals are permanent reservoirs for infection due to lack of treatment; therefore, it is necessary to stop the transmission chain for prevention and infection control. An important step to achieve this goal is to identify infections in asymptomatic and symptomatic patients (6).

In addition to adverse social effects, the cost of treatment is high. The annual cost of treating the complications caused by *C. trachomatis* infections in the United States is estimated to be over \$ 2 million (5).

C. trachomatis specie has 19 serotypes (K-A, L1, L2, L2a, L3, Ba, Ga, Da, and Ia) that are identified by polyclonal and monoclonal antibodies against the outer membrane protein (MOMP) (7).

MOMP is coded by the *omp1* gene and is the immunodominant antigen of *C. trachomatis* that acts as a cytoadhesion by facilitating the interaction between bacteria and host cells. It

has been proven that MOMP is also involved in adhesion mechanisms and pathogenesis (8). For many years, isolation of bacteria in tissue culture usually adjacent to McCoy cells or cyclohexamide was the method of choice for detection of *C. trachomatis* (9).

However, this method requires accurate sample collection and transfer conditions and 48 to 72 hours to run (2). The molecular genetic techniques are useful for identification of microorganisms that are difficult to cultivate or grow slowly (such as *C. trachomatis*) (10).

Recently, the polymerase chain reaction (PCR) method is used for the diagnosis of *C. trachomatis* infections. The previous studies show that PCR method has 97% to 100% sensitivity and 98% specificity for the detection of *C. trachomatis*, while cultivation has 85% and 100% sensitivity and specificity, respectively (2). The DNA amplification techniques are used for urine samples in addition to cervical and vaginal swabs (11).

A compiled program to recognize the status of the pathogen in different social groups, to identify risk factors predisposing to the infection and to perform screening programs can reduce the burden of this disease in our society.

Considering the complications of chlamydial infection in women, this study aimed to determine the prevalence of infection in symptomatic and asymptomatic women using a sensitive molecular method.

MATERIAL AND METHODS

This study was conducted on 150 non-pregnant women referring to gynecological clinic of SayyadShirazi Hospital in Gorgan.

The participants were categorized in the group with genital symptoms based on physical examination by a physician and questionnaire information, if they had at least one or more of the following clinical symptoms: abnormal vaginal discharge, spotting, bleeding after intercourse, pain or burning during urination, lower abdominal pain and painful intercourse (dyspareunia). The women with none of the mentioned symptoms, admitted for other reasons, were placed in the asymptomatic group.

The inclusion criteria were marriage, reproductive age and nulliparity. The eligible placed in the asymptomatic group.

The inclusion criteria were marriage, reproductive age and nulliparity. The eligible individuals were recruited in the study after receiving brief explanation about the study. After obtaining written consent from all participants, they were interviewed and completed a previously designed questionnaire consisting of information about age, ethnicity, and type of clinical symptoms indicative of infection.

The samples were obtained by cervical swab and kept in 2 ml of phosphate-buffered They were then quickly transferred to the Microbiology laboratory of Golestan University of Medical Sciences on the same day.

To extract the DNA ,two methods of phenol-chloroform and boiling were used.

One ml of the sample solution was centrifuged at 8000 rpm for 10 minutes. The supernatant was removed and 600 µl of lysis buffer (EDTA-NaCl, Tris), 13 µl 10% SDS and 5 µl proteinase K were added to the sediment. The sample was kept at 60 °C for one hour.

Phenol-chloroform at the same volume of sample solution was added to DNA lysate and then slowly mixed. The mixture was centrifuged at 10000 rpm for 10 minutes and then supernatant was transferred to a new microtube.

Next, 500 µl of 7.5 M sodium acetate and 2 ml of absolute ethanol were added and the tube was place at -70 °C for 10 minutes. Then, it was centrifuged again for 30 minutes at 10000 rpm.

The supernatant was removed and the sediment was washed with 70% ethanol and the microtube was placed to dry up. Finally, 10-50 µl of the distilled water were added to the mirotube and then it was kept at -20 °C until the PCR experiment.

First, the solution containing samples was

vortexed and 400 µl transferred to a microtube. The microtube was centrifuged at 13000 rpm for 30 minutes. The supernatant was removed and 100 µlTris-Hcl (10 mM) with pH7.5 added to the sediment. The microtube was stored at -70 °C for 48 hours.

After melting, it was boiled for 10 minutes at 100 °C and then the mixture was centrifuged for 2-6 minutes at 10000 rpm. The supernatant containing DNA was transferred to another microtube and kept at -20 °C until the PCR experiment. Quality and quantity of the extracted DNA through both methods were assessed using spectrophotometry and electrophoresis on agarose gel (1%).

PCR process The following *omp-1*-specific primers were used for the PCR (12, 13):

omp1-F: 5'-GCC-GCT-TTG-AGT-TCT-GCT-TCC-TC-3'

omp1-R: 5'-ATT-TAC-GTG-AGC-AGC-TCT-CTC-AT-3'

PCR was performed using Bioflux kits at volume of 50 µl. The PCR buffer (10X), MgCl₂ (50mM), dNTP (10mM), forward and reverse primers at concentration of 30 pm, Taq DNA polymerase (1U/µl) and deionized distilled water were used. The PCR programs included five-minutes at 95 °C, 1 minute at 95 °C, 1 minute at 55 °C and 90 seconds at 72 °C over 40 cycles.

The PCR products were electrophoresed on 1.5% agarose gel stained with ethidium bromide, and then examined under UV light. Along with each PCR run, a standard positive control sample (F/IC-CAL3) and negative control were included to verify the accuracy of the PCR run.

RESULTS

The age range of the participants was 18 to 60 (mean age 37). The demographic and clinical characteristics are presented in Table 1.

Table 1- Demographic and clinical characteristics of subjects

		Subjects															
		Reproductive capability		Cervicitis		Type of symptom						Symptom		Ethnicity			
		-	+	-	+	Dyspareunia		Lower abdominal pain	burning during urination	Bleeding after intercourse	Spotting	Abnormal discharge	-	+	Sistani	Turkmen	Fars
%		2.7	97.3	86	14	34.7	45.3	24	11.3	15.3	46	23.3	76.7	28	2.7	69.3	

DISCUSSION

C. trachomatis is one of the most common causes of STDs in the world (3). Since 50% of infected men and 80% of infected women are asymptomatic, the actual number of reported cases represents only part of the population infected with *Chlamydia*, which can make detection and diagnosis difficult (7). On the other hand, lack of timely diagnosis and treatment can lead to ascending infections, causing inflammation and scarring in the reproductive system of men and women. Many reports have shown that these infections are the major cause of pelvic inflammatory disease (PID) in women. One of the most important complications of PID is infertility due to obstruction of fallopian tubes, ectopic pregnancy and chronic pelvic pain (14). In many countries, the incidence of ectopic pregnancy is increasing, which is the main cause of maternal mortality in the first trimester of pregnancy. In addition, *Chlamydia* can be transmitted to the baby at birth and leads to pneumonia and conjunctivitis (14). The untreated men are also at risk of urethritis, proctitis, epididymitis or epididymo-orchitis (3). In addition to high cost of treatment, the complications of chlamydial infection can sometime lead to some social problems in families. Since the symptoms are not specific to *C. trachomatis* infections, the definitive diagnosis of this infection requires laboratory methods (15). For this purpose, the present study assessed the frequency of *C. trachomatis* in symptomatic and asymptomatic women. Since this was an epidemiological study, the choice of sensitive diagnostic method for determining the prevalence was very important. Several studies have been conducted in recent years in order to find the best method of diagnosing chlamydial infections. Since *Chlamydia* is an intracellular pathogen, its identification using routine methods of bacteria detection is difficult. Several studies have shown that urine and swab samples can be used for screening *C. trachomatis* as the gold standard for diagnosing chlamydial infections, due to the high sensitivity and specificity of DNA amplification techniques. In addition to low cost of these techniques for testing a large number of samples, it is sensitive enough to

a small number of bacteria, PCR with high sensitivity is capable of detecting even small number of bacteria and determining the prevalence of infection in these individuals (17). Several studies in other countries investigated the prevalence of chlamydial infections using different methods by considering their sensitivity and specificity. Goulet study on the general population of France, aged 18-44, reported the prevalence of this infection 1.4% in men and 1.6% in women using the PCR method (18). Using the same method, the frequency of *C. trachomatis* in endocervical samples was 9.2% in India (20). The highest rate of infection (27%) was observed in New Guinea (21), while its prevalence was reported 1.1% in Joyee study in India on urine samples of women using PCR (22). The results of the present study are in agreement with Deeb et al. study. Although in this study the prevalence of other STDs was 1.2%, no positive case of chlamydial infection was found (23). Studies of different groups in Iran reported different frequencies for these infections. In a study in Tehran, the prevalence of *C. trachomatis* in symptomatic and asymptomatic women was reported 14.99% (24). While, another study on women aged 15-42 in Tehran reported that 12.8% had positive PCR test (16). Moreover, a study reported that 13.2% of women with spontaneous abortion were infected with *C. trachomatis* (5). The prevalence in Ahwaz was 18.1% in a study on cervical samples from symptomatic women using the *omp1* gene (12). In the study conducted by Nasrollahi, the prevalence in asymptomatic and symptomatic women was 16% and 45.2%, respectively (25).

In Kalantar et al. study in Yazd, no positive cases of *C. trachomatis* infection was found among blood and vaginal samples of infertile women using PCR and ELISA methods (15).

The difference between these results could be due to diagnostic methods, different sample type and methods of processing and preparation (26). In this study, the highly sensitive PCR method was applied using *omp1* gene to obtain reliable results. Two methods of phenol-chloroform and boiling were used for DNA extraction from cervical swab samples but no positive case of *C. trachomatis* infection was found.

detect *Chlamydia* (16). Given that asymptomatic infections are accompanied with

CONCLUSION

Considering the serious complications of chlamydial infections and its asymptomatic nature, a highly sensitive and specific method such as PCR should be used to detect

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Chlamydia.

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CONFLICT OF INTEREST

The authors have no conflict of interests.

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