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

**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.
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 soc-
arious 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 pre-
valence 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

INTRODUCTION
Chlamydia trachomatis is a small
cocxoid, 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
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 saline (PBS) solution. 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 400 μl transferred to a microtube. The microtube 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 μl Tris-Hcl (10 mM) with pH 7.5 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
omp1-R: 5’-ATT-TAC-GTG-AGC-AGC-TCT-CTC-AT-3’

PCR was performed using Bioflux kits at a 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>
<thead>
<tr>
<th>Subjects</th>
<th>Reproductive capability</th>
<th>Cervicitis</th>
<th>Type of symptom</th>
<th>Symptom</th>
<th>Ethnicity</th>
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<td>Dyspareunia</td>
<td>Lower abdominal pain</td>
<td>burning during urination</td>
<td>Bleeding after intercourse</td>
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<td>2.7</td>
<td>97.3</td>
<td>86</td>
<td>14</td>
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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 find the best method of diagnosing 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 Joyce 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 chlamydiyal infections and its asymptomatic nature, a highly sensitive and specific method such as PCR should be used to detect *Chlamydia*

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**CONFLICT OF INTEREST**

The authors have no conflict of interests.

**REFERENCES**


In another study, the frequency of *C. trachomatis* in women aged 15-24 in Brazil was 9.8% using PCR (19).


