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Frequency of *Chlamydia trachomatis* in Endocervical Samples of Women Referred to a Gynecology Hospital in Qazvin, Iran

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Background: *Chlamydia trachomatis* (*C. trachomatis*) is the most common bacterial sexually transmitted infection (STI). Although most genital *C. trachomatis* infections remain asymptomatic but infection with these bacteria is the leading cause of complications, such as pelvic inflammatory disease (PID), tubal factor infertility and abortion.

Objectives: The objective of this study was to estimate the frequency of *C. trachomatis* infection among symptomatic and asymptomatic women, by a polymerase chain reaction (PCR) based assay.

Patients and Methods: This was a cross-sectional study conducted over the period from May 2012 to February 2013. A total of 240 non-duplicate endocervical samples were taken from married women; endocervical swabs were collected from women referred to Qazvin Kowsar Gynecology Hospital by a training midwife. The specimens were tested for *C. trachomatis* by a PCR-based assay for the *pha* gene.

Results: Out of the 240 female participants, 184 (76.7%) were symptomatic and 56 (23.3%) were asymptomatic cases. The mean age of cases was 37.1 ± 0.9 years. Twenty (8.3%) of the 240 samples were diagnosed as *Chlamydia* positive according to PCR results. The prevalence of asymptomatic *C. trachomatis* infections was six (10.7%), while there were 14 (7.6%) in symptomatic cases. Although positive PCR results have shown in women with vaginosis (7.1%), abortion (5.1%), premature birth and low birth weight below 2.5 kg (7.7%) but the chi-square test did not indicate a significant relationship between positive PCR test results and these symptoms.

Conclusions: The results of this study showed that there was a high prevalence of *C. trachomatis* infection among both symptomatic and asymptomatic women. Therefore, a screening test for *C. trachomatis* infection is recommended for all women who refer to the genitourinary medicine clinic. Screening programs are important for cost effectiveness calculations of *C. trachomatis* infections especially in asymptomatic cases.

Keywords: Chlamydia trachomatis; Women; PCR; Iran

1. Background

Chlamydia trachomatis (*C. trachomatis*) is an obligate intra-cellular pathogen and the most frequent sexually transmitted bacterium worldwide (1-4). Most infections occur without symptoms and asymptomatic manifestation of urogenital chlamydial infection can be observed in ~30% of patients. Although effective antimicrobial therapy is available for *C. trachomatis*, yet undetected and untreated infections lead to diseases such as salpingitis, pelvic inflammatory disease (PID), tubal infertility and abortion (5-9). *Chlamydia trachomatis* in the cervix could be transmitted to a neonate during passage through an infected birth canal, resulting in neonatal pneumonia (6, 10).

The gold standard for diagnosis of *C. trachomatis* infection has traditionally been a culture of swab from the endocervix in women or the urethra in men (6). However, the methodological challenges of culturing of this organism and difficulty of this method have led to the development of non-culture-based tests, including antigen-detection tests and nucleic acid hybridization. These techniques however, fail to detect a substantial proportion of infections. Newer tests are accordingly required to be developed that could amplify and detect *C. trachomatis*-specific DNA or RNA sequences, with greater sensitivity than first generation non-culture-based tests (10-14). In many studies, the target gene for nucleic acid amplification tests (NAATs) has been the cryptic plasmid. Some studies give evidence or suggest that plasmid-free variants are present in clinical samples. Thus, infections caused by plasmid-free variants will be undetected if the plasmid is used as the target gene (12). Targets for amplification have included both cryptic plasmid (7, 8, 10, 18, 19, 22 - 24, 27) and chromosomal genes, including those for the major outer

membrane protein (MOMP) (2, 11, 23, 25, 33), *gyr*A and phospholipase D endonuclease superfamily gene (PRPHA).

2. Objectives

The objective of this study was to estimate the frequency of *C. trachomatis* infection among symptomatic and asymptomatic women, by a polymerase chain reaction (PCR)-based assay using unique sequences (15).

3. Patients and Methods

This was a cross-sectional study conducted over the period from May 2012 to February 2013. During a ten-month-period, 240 endocervical samples were taken from married women, who had referred to Kowsar Gynecology Hospital Qazvin, by a training midwife. The patient's demographic data and medical histories were collected by direct interviews and completion of the questionnaire.

3.1. Sample Collection

Briefly, cervical mucus was removed prior to insertion of a Dacron swab into the endocervical canal; the swab was then immersed in 1 mL of phosphate buffered saline (PBS) transport medium. All PBS media were maintained at 4°C during specimen collection and then aliquoted into DNase and RNase free microtubes and frozen at -80°C within four hours of collection until DNA extraction (16).

3.2. DNA Extraction

DNA was extracted from endocervical samples using the boiling technique by DNG PLUS (CinnaGen, Iran). The concentration of DNA samples was determined as micrograms per milliliter based on A260 values and adjusted to 1 μL/mL prior to PCR amplification. All extracted DNA were stored at -20°C until analysis by PCR.

3.3. Detection of Chlamydia trachomatis by PCR

PCR assay was performed for detection of *C. trachomatis* by the *pha* gene. The primers were F-(5'TCTTTTTAAACCTC CGGAACCCACTT3') and R-(5'GGATGGCATCGCATAGCATTCT TTG3'). The PCR product size was 378 bp. PCR amplification was performed in a final volume of 20 μL, containing 1 μL of each primer (10 pmol/μL, 10 μL master mix sybr green (Bioneer-USA)), 6 μL of distilled water and 2 μL of DNA samples. Sequenced *C. trachomatis* DNA was used as the positive control and distilled water was used as the negative control. DNA amplification was carried out with the following thermal cycling profile for *pha* gene; 10 minutes of initial denaturation at 95°C followed by 35 cycles of amplification. Each cycle consisted of denaturation at 95°C for 15 seconds, annealing at 60°C for 30 seconds, and extension at 72°C for 30 seconds. The PCR products were analyzed by 1.5% agarose gel electrophoresis and the product size was estimated using a 100 bp DNA ladder (Fermentas).

3.4. Statistical Analyses

Data Statistical analysis was performed using the Statistical Package for the Social Sciences software (SPSS) (version 19.0; SPSS Inc, Chicago, IL, USA). Data were summarized by frequencies and percentages for categorical variables and by means and standard deviations for normally distributed data. The relationship between results was analyzed by chi-square tests. A P value of < 0.05 was considered as statistically significant.

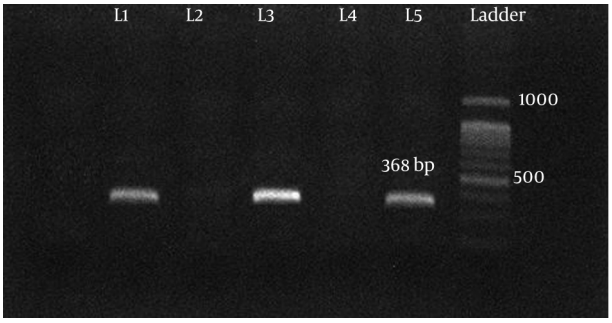
4. Results

The study population consisted of 240 married women; 184 (76.7%) symptomatic and 56 (23.3%) asymptomatic cases. The mean age of cases was 37.1 ± 0.9 years. During the period of this study, 240 married women with various complications were enrolled, including: 18 (7.5%) infertility, 58 (24.16%) abortion, 126 (52.5%) vaginosis (signs and symptoms included vaginal discharge, vaginal irritation and dysuria), 13 (5.4%) premature birth, 15 (6.25%) and low birth weight below 2.5 kg. Out of the 240 endocervical swab samples, 20 (8.3%) were positive for *C. trachomatis* by PCR of *pha* gene (Figure 1). Of the 56 asymptomatic women, six (10.7%) had *chlamydia* infection while among the 184 symptomatic cases 14 (7.6%) had this infection, according to the PCR results.

Among symptomatic women with chlamydial infection, infertility was not present in any of the cases (0%), while abortion was present in three (5.1%), vaginosis in nine (7.1%) premature birth in one (7.7%) and low birth weight in one (7.7%).

The chi-square test was used to evaluate the relationship between symptoms of genital infection using PCR. None of the symptoms such as genital discharge, dysuria, genital itching (vaginosis), abortion, premature birth, low birth weight (below 2.5 kg) and infertility showed a significant association with the infection (Table 1).

Figure 1. Detection of *Chlamydia trachomatis* From Endocervical Swab Specimens by PCR Using *pha* Gene Primers



100 bp DNA ladder L2, L4: negative clinical sample; L1, L3: positive clinical sample; L5: sequenced positive control

Table 1. Frequency of *Chlamydia trachomatis* in Endocervical Specimens of Married Women According to Symptoms[a]

Characteristic	PCR Positive	PCR Negative	Total	P Value
Symptomatic				
Infertility	0 (0)	18 (100)	18 (100)	0.36
Abortion	3 (5.1)	55 (94.9)	58 (100)	0.55
Vaginosis	9 (7.1)	117 (92.9)	126 (100)	076
Premature birth	1 (7.7)	12 (92.3)	13 (100)	1.0
Low birth weight below 2.5 kg	1 (7.7)	12 (92.3)	13 (100)	1.0
Asymptomatic				
Healthy women	6 (10.7)	50 (89.3)	56 (100)	

[a] Data are presented as No. (%).

5. Discussion

Urogenital *C. trachomatis* infection has become a major public health problem world-wide and is one of the most frequent causes of sexually transmitted diseases (1, 2). It is a common cause of urethritis and cervicitis. Some reported sequelae include PID, abortion, tubal factor infertility, epididymitis, proctitis and reactive arthritis (5-9). The majority of *chlamydia*-infected individuals are asymptomatic, and remain unnoticed and untreated. Infected women may be at risk of gynaecological complications (e.g. PID and tubal infertility), and determine the reservoir for onward transmission in the population (17).

In the current study, the prevalence of *C. trachomatis* infection among 240 women attending Kowsar Gynecology Hospital in Qazvin was 8.3%; six cases were asymptomatic (10.7%), while 14 (7.6%) were symptomatic. The chi-square test did not show a significant relationship between positive test results and vaginosis, abortion, premature birth, low birth weight below 2.5 kg and infertility.

Torrone et al. (18) reported that the prevalence of *C. trachomatis* genital infection among people aged 14-39 years in the United States was 1.7%, during 2007-2012. Overall the prevalence of chlamydial infection among individuals aged 14-39 years was similar over the three National Health and Nutrition Examination Survey (NHANES) cycles combined for this analysis: 2007-2008: 1.6% (CI = 1.1-2.2%); 2009 - 2010: 1.7% (CI = 1.2-2.1%); and 2011-2012: 1.9% (CI = 1.5-2.2%). *Chlamydia* is the most commonly reported nationally notifiable disease, with over 1.4 million infections reported in 2012. However, case reports commonly underestimate the burden of this disease because most infections are asymptomatic and are neither diagnosed nor reported. The 2007-2012 NHANES indicated that an estimated 1.8 million people aged 14-39 years in the United States have a genital chlamydial infection. These results showed increasing rates of chlamydial infection during 2007-2012 in the USA (18). Based on several investigations, prevalence of *C. trachomatis* infection varies with different populations and the type and sensitivity of the detection methods used.

Marcone et al. (19) reported the prevalence of *C. trachomatis* infection of women in Rome. The mean prevalence of *C. trachomatis* endocervical infection during 2000 to 2009 was 5.2%. The results of this study showed a high rate for sexually transmitted infections (STI). Al-Thani et al. (9) reported the prevalence of *C. trachomatis* infection among 377 (37.9% Qataris and 62.1% non-Qataris) healthy women attending primary healthcare centers in Qatar. The specimens were tested for *C. trachomatis* by a PCR-based assay. Prevalence of *C. trachomatis* infection was 5.3% among Qatari women and 5.5% among non-Qatari women. Similar to our study, this high prevalence may reflect, in part, the limited access to and use of *chlamydia* screening and management (9). Araujo et al. (20) in central Brazil reported that the overall prevalence of *C. trachomatis* infection by PCR was 19.6%. Cuffini et al. (21) reported that the prevalence of genital *C.* trachomatis infection in asymptomatic women in Argentine was 13.7%. Their results showed higher prevalence of *C. trachomatis* infection among Latin-American women than our study.

The present study showed that *C. trachomatis* endocervical infection could be present in symptomatic (7.6%) as well as asymptomatic women (10.7%). The majority of *chlamydia*-infected individuals were asymptomatic, and remained undetected, unnoticed and untreated. Infected women may be at risk of gynecological complications (e.g. PID and tubal infertility), and determine the reservoir for onward transmission in the population. Strategies for the control of infection and prevention of its complications are only partially effective because there is no available vaccine while there is high-risk sexual behavior in the population. Screening programs have been introduced as an additional strategy for early detection and treatment of infected cases. Cost-effectiveness of screening is largely determined by the rates of complications prevented. Nucleic acid amplification methods, such as PCR, have high sensitivity, specificity and require a short duration of time for obtaining results, therefore are preferred for diagnosis of chlamydial infection.

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Authors' Contributions

Study concept and design: Masoumeh Aslanimehr. Acquisition of data: Saman Saadat. Analysis and interpretation of data: Masoumeh Aslanimehr. Drafting of the manuscript: Mehry Sadeghi Ghazvini. Critical revision of the manuscript for important intellectual content: Masoumeh Aslanimehr. Statistical analysis: Amene Barikani. Administrative, technical, and material support: Taghi Naserpour Farivar. Study supervision: Masoumeh Aslanimehr.

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