

Prevalence of *C. Trachomatis* Infection among Ahvaz Females with Vaginal Discharge

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Abstract

Background: *C. trachomatis* is one of the most important agents of sexually transmitted diseases (STD). Untreated cases of vaginal infection with this bacterium may end up with infertility, scarring and ectopic pregnancy. In most cases infection with *C. trachomatis* is silent (70-80%) but when overt infection occurs the symptoms include: cervicitis, urethritis, and endometritis.

Since prevalence of *C. trachomatis* was not studied in our city, this project was performed to evaluate the role of this bacterial agent in vaginosis.

Material and Methods: Two hundred and two specimens from vaginal discharge of out-patients referring to gynecology ward of Ahvaz hospitals were collected and tested for *C. trachomatis* by PCR.

Results: Out of 202 specimens, 33 (16.3%) were positive for *C. trachomatis*.

Conclusion: The findings of this study show a high prevalence of *C. trachomatis* which indicates that special attention must be paid to detection and management of this infection.

Keywords: *C. trachomatis*, Vaginal Discharge, Vaginosis

Introduction

Chlamydiae are non-motile gram negative obligatory intracellular pathogenic parasites. In their life-cycles, these bacteria make intracellular inclusion bodies that are visible under light microscope. The genus *Chlamydia* includes 4 species, among which *C. trachomatis* is the etiologic agent for trachoma and urogenital infection. According to various investigations, *C. trachomatis* is the most prevalent cause of sexually transmitted disease in USA (1, 2) and probably in the world (3). According to WHO, 90 million new cases of chlamydial genital infection are reported annually (4).

The most common risk factor for acquiring genital chlamydial infection is low age of females (<20 years). Although most chlamydial infections in females are silent, cervicitis, urethritis, endometritis, pelvic inflammatory disease (PID) and inflammation of Bartolene glands are common clinical manifestations of symptomatic cases (4). Asymptomatic *C. trachomatis* infections are major causes of PID and ectopic pregnancy. Moreover, asymptomatic and untreated salpingitis is the significant cause of infertility in females (5).

Although *C. trachomatis* infections may be asymptomatic, the immunological damages to fallopian tubes are more severe than the damages introduced by any other bacterial species (6).

Laboratory diagnosis of *C. trachomatis* is based on culture of the specimens on the cell culture media, serological diagnosis of anti-chlamydial antibodies, indirect fluorescent antigen tests and molecular techniques such as PCR. Among these, PCR is the most sensitive and specific method for laboratory diagnosis of this bacterium. Since the specimens for PCR are collected non-invasively, this method is easily accepted by the patients as well. In this project, the prevalence of *C. trachomatis* was determined by PCR among females with cervical discharge who referred to Ahvaz Imam Khomeini, Golestan and Amir-almomenin hospitals as well those admitted to some private gynecologic clinics during the years 2001 through 2003.

Material and Methods

The cases included in this study were females with vaginal discharge admitted to one of

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hospitals in Ahvaz for treatment.

Detection of urinary infection was based on positive results of urine culture reported by hospital laboratories.

Specimens of vaginal discharge were collected by gynecologists.

All buffers and reagents used in this project were prepared with deionized water. The chemicals were purchased from Merck (Germany) and PCR components such as primers, dNTPs, *Taq* DNA polymerase were purchased from Roche (Germany). Two hundred and twelve vaginal discharge specimens were collected from the patients with sterile cotton swabs. Each swab was placed into a tube containing 2 mls of 0.1 M Phosphate buffered saline (PBS) supplemented with 0.2 M sucrose and transported to the laboratory. The specimens were either used immediately for DNA extraction or saved at -20° C till performing the experiment. An information sheet including the age of the patient, socioeconomic and marital status, history of PID and abortion and nulligravidity was filled for each case.

Extraction of *C. trachomatis* DNA was carried out by phenol-chloroform method (7). Briefly, the sample tube was centrifuged at 5000 rpm for 15 minutes and the sediment was dissolved in homogenizing buffer (25mM Tris-HCl, 10mM EDTA, 100mM NaCl, 0.5% SDS) and transferred to a 1.5 ml microfuge tube. To each microtube 1ml buffered phenol initially warmed at 65° C was added and centrifuged at 12000 rpm for 15 minutes. Following centrifugation, the supernatant was collected and transferred to a fresh microtube. This step was performed twice and 0.5 ml chloroform-isoamyl alcohol (24:1) was added to the supernatant, mixed well and centrifuged at 12000 rpm for 15 minutes. The supernatant was collected and 3 volume of absolute ethanol was added to it and freezed at -20° C for at least 3 hrs. Then the tubes were picked and centrifuged at 12000 rpm for 15-30 minutes. Ethanol was suctioned and the tubes were left opened at room temperature for a half an hour to air dry completely. The pellets were dissolved in 50 µl sterile deionized water. Concentration and purity of DNA was determined by spectrophotometry at 260nm and 280nm. The DNA was saved at -20° C to be used as template of PCR reaction (7). The primers used in this experiment were

recommended by Roche company and their sequences were as follow: KL1 5'- TCC GGA GCG AGT TAC GAA GA-3' and KL2 5'- AAT CAA TGC CCG GGA TTG G-3'. The length of the amplicon was 241bp from a *C. trachomatis* plasmid. The volume of PCR reaction was 50µl and ingredients of PCR reaction were 5µl 10x PCR buffer, 2µl dNTPs (200mM each), 5µl each primers, 4µl 2mM MgCl₂, 0.5µl 5 unit/µl *Taq* DNA polymerase, 5µl template and 23.5 µl H₂O. Twenty to 30 µl mineral oil was added to each tube. PCR conditions were 94° C for 3 minutes as initial denaturation T_m followed by 30 cycles of 94° C for 1 minute, 55° C for 1 minute and 72° C for 1 minute. For final extension, the PCR machine was programmed to stop at 72° C for 10 minutes. PCR machine used in this project was Eppendorf Mastercyle 5330 (Germany). Ten µl of PCR products were subjected to electrophoresis in 1% agarose gel in TBE buffer and stained with 1 µg/ml ethidium bromide and visualized under UV-trans-illuminator (Fig 1). For positive control, DNA of *C. trachomatis* cultured on egg yolk was extracted by phenol-chloroform and 5µl of DNA was applied in positive reaction tube as template. In negative control reaction, 5µl H₂O was used as template. SPSS software and X² test was used to evaluate the relationship between risk factors and laboratory results.

Results

Of 212 collected vaginal discharges, 10 samples were omitted from the experiment for various reasons such as shortage of specimen or contamination due to poor handling, thus PCR was performed for 202 remaining samples. Of these specimens, 33 (16.3%) were positive for *C. trachomatis*. Statistical analysis of the results showed no correlation between positive cases and risk factors (table 1-3).

Table 1: Relationship of age with PCR results

AGE	PCR Negative	PCR Positive
<36	69	21
>35	73	12

P = 0.11

Table 2: Relationship of patients with sexually transmitted diseases with PCR results

PCR Negative	PCR Positive
154	30
12	30

P = 0.46 (Fisher exact)

Table 3: Relationship of urogenital infections with PCR results

Urogenital Infection	PCR Negative	PCR Positive
Negative	97	20
Positive	71	13

$P=0.76$

Besides, the infection was higher in the age group 20-35 years old compared to older cases (10.4% versus 5.9%). In our study there was a strong correlation between positive cases and some of risk factors such as the age of the cases.

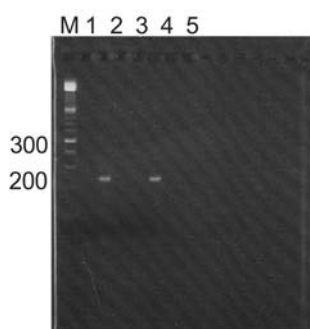


Fig 1: Agarose gel electrophoresis of *C. trachomatis* PCR products. Lane 1: DNA ladder (Roche DNA molecular marker XIV). Lane 2: Negative Control. Lane 3: Positive Control. Lane 4: Positive sample.

Discussion

The prevalence of *C. trachomatis* infection varies in different societies. For example Klaves and his colleagues examined vaginal discharge of females aged between 18-24 years for *C. trachomatis* in Slovenia. According to their report, the rate of this infection was 1.6% which is considered very low (8). In a similar research performed by Malano and colleagues, this figure was as high as 35% in females of Jamaica (9). The rate of *C. trachomatis* infection was 3% in Netherlands (10), 4.5% USA (11), and Finland (12), 9% in Colombia (13) and 11-11.5% in females of Central America and north of Australia (14). Also Katz conducted a study on imprisoned women in Honolulu, Hawaii. The prevalence of *C. trachomatis* in his sample group was 6.3% (15).

Previous studies conducted on male cases in Tehran (16) and prostitutes of Bandar Abbas (south of Iran) (17), revealed the prevalence of *C. trachomatis* to be 2.75% and 12.38%, respectively. The method of detection employed in both studies was based on

examination of sera and cervical discharges by immunofluorescence, a method that is not as sensitive as PCR.

Our research demonstrated that the prevalence of *C. trachomatis* was 16.3% which is relatively higher than previous studies and suggests possible under-estimation of the prevalence of this bacterium in those reports.

Results (16.3%), obtained from our study was higher than we suspected. Many factors may have influenced the rate of infection among Iranian females living in Ahvaz. Due to anatomical variations, younger women are more susceptible to *C. trachomatis* infection compared with older ones. Besides, young groups are more sexually active than elders which in turn elevate the chance of spread of bacteria.

Poor hygienic practices are another factor that increases the chance of bacterial infection. For this reason *C. trachomatis* infection is higher in lower socio-economical classes.

The relationship of other variables such as economical status, history of abortion or ectopic pregnancy, and color of discharge was determined and found non-significant (data not shown). Many factors can be listed as the cause of this discrepancy. For example, some of the cases did not declare their level of income or were reluctant to fill this item, although this information was gathered by similar studies in other societies such as Bogotá, Colombia (9).

In India, 143 females who attended an STD center were monitored for *C. trachomatis* infection by culture of the specimens and direct fluorescent antigen test (DFA). The prevalence of infection was 25%. But again, there was no statistically significant relationship between risk factors and positive results (18).

Even *C. trachomatis* infection rate was not significantly related to sexual activity and multiple sex partners, as shown by a study conducted by Shields on female sex workers in Canada (19).

In this project, the prevalence of *C. trachomatis* infection was determined by PCR. The sensitivity and specificity of PCR for detection of this bacterium has been reported 98% and 100% respectively (20). Using this method it was revealed that the prevalence of infection with this bacterium (16.3%) was considerably high in our study group. Since

only the cases with vaginal discharge were included in this study, and because most chlamydial infections are asymptomatic, the real prevalence of infection with this bacterium could be higher.

Chlamydial infection is related to PID, prehepatitis, endometritis, and infertility. Chronic infection with this bacterium might lead to cancer of cervix. Particularly because most *C. trachomatis* infections are silent (85-90%) (21), laboratory detection of this bacterium, especially in females, and subsequently, report of the results to physicians for treatment may result in dramatic decrease of the rate of infertility and cervical neoplasia (22).

Conclusion

As a conclusion it should be noted that results of this study were obtained from a limited number of a selected population of females who had vaginal discharge. Therefore, generalization of these findings to the whole population is not logical and suggests the need for further studies to elucidate the prevalence of this agent in a larger population of asymptomatic females.

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