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Chlamydia trachomatis Detection by Nested-PCR Method on Females Referred to Medical Centers of Tehran, Iran

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Background: *Chlamydia trachomatis* (*C. trachomatis*) is the most common sexually transmitted bacterial infectious disease in the world. Moreover, it plays a role in spontaneous abortion. The accuracy of PCR in detection of *C. trachomatis* infections has been shown in several studies.

Objectives: The frequency of spontaneous abortion and known side effects and statistics vary in *Chlamydia trachomatis* infection in women with spontaneous abortion and different ways to identify and determine the prevalence of *Chlamydia trachomatis* are used.

Materials and Methods: Four sterile Dacron swabs were used to collect specimens from endocervix and vagina from women with miscarriage. DNA was extracted by AccuPrep Genomic DNA extraction kit. The nested PCR procedure was performed with two pair primers. This study was conducted on women referred to Medical Centers of Tehran, Iran in 1391.

Results: The number of intercourses per week and history of miscarriage can be known as the risk factors of abortion. Frequency of *C. trachomatis* in endocervix was 13.25%; the amount of vaginal infection among this group was 19%.

Conclusions: Nested PCR as a sensitive *Chlamydia trachomatis* detection test and endocervical specimens has been offered to detect this bacterium in spontaneous abortion. Besides, *C. trachomatis* screening among pregnant women can be suggested to prevent abortion.

Keywords: Chlamydia trachomatis; Abortion, Spontaneous; Polymerase Chain Reaction

1. Background

One of the appropriate anatomical places for the growth of many micro-organisms in woman body is the genital tract. *Chlamydia trachomatis*, *Ureaplasma urealyticum*, *Gardnerella vaginalis*, *Listeria monocytogenes* and *Neisseria gonorrhoeae* are some of the bacteria localize in female genital tract (1). *Chlamydia trachomatis* (*C. trachomatis*) is the most causative agent of bacterial sexually transmitted infections (STI) (2, 3). There are about 90 million cases with STI throughout the world, annually (3). It can cause cervicitis, pelvic inflammatory disease (PID), and urethritis in women (4). However, most of contaminations with *C. trachomatis* remain undetectable (70% - 80%), because these cases are often asymptomatic (5). *C. trachomatis* in pregnant women increases the risk of ectopic pregnancy, spontaneous abortion, premature rupture of membranes, postpartum endometritis, perinatal mortality and low birth weight (6). Moreover, pathogenesis of *Chlamydia trachomatis* in abortion has been determined (2, 7).

C. trachomatis is a gram negative, pleomorphic, intracellular and nonmotile organism with about 0.2 - 1.5 μm length (8, 9). *C. trachomatis* detection tests are divided in two groups; cell culture and non-cultural tests. Cell culture facilities do in specialized research laboratories only. Non-cultural cells such as enzyme immunoassays (EIAs), serological tests have no enough accuracy, sensitivity and specificity to diagnosis of *C. trachomatis* infections (10). Polymerase chain reaction (PCR) and ligase chain reaction (LCR) have been offered for detection of *C. trachomatis* (10, 11). Accuracy of nucleic acid amplification tests (NAATs) in detection of *C. trachomatis* infections have been shown in several studies (11, 12). The specificity of the PCR was 100% and the sensitivity was 100% (13).

2. Objectives

The aim of this study is to evaluate the *C. trachomatis* infection frequency by nested-PCR method among women with abortion in Tehran, Iran.

Implication for health policy/practice/research/medical education:
The study is conducted for medical educational purposes.

3. Materials and Methods

Specimen collection: from November 2011 to September 2012, 121 women were enrolled in this study; they had been referred to medical centers, Shahid Beheshti University of Medical Sciences, Tehran - Iran. Questionnaires were filled for each person. By using speculum, 4 sterile Dacron swabs were used to collect specimens from endocervix and vagina. Two Swabs were placed in 0.2 M sucrose phosphate (2 SP, pH = 7.2) buffer tubes with 10% fetal bovin serum, antibiotics (Streptomycin 50μg/mL + Ancomycin 100 μg/mL + Gentamicin 10μg/mL; Sigma Co.) (14) and two swabs for other bacteria. All specimens were transported on ice to the microbiology research laboratory of Shahid Beheshti University. All specimens were stored at -70°C until DNA extraction.

DNA extraction: DNA extraction was performed by Accu-Prep Genomic DNA Extraction (Bioneer Co., Korea). Specimens were thawed; 20 μL Proteinase K and 200 μL binding buffer were added to 200 μL of samples, after mixing, they were incubated at 60°C for 20 minutes. 100 μL isopropanol was added to mixtures. Lysates were centrifuged at 8000 rpm for 1 minute; washing buffer 1 and then 2 were added to tubes, respectively. After centrifuge, 200 μL elution buffer was added and centrifuged at 8000 rpm for 1 minute, finally. Eluted genomic DNA stored at -20°C for further analysis. PCR: For preparation Master mix (1 X); 11.6 μL PCR Master mix was added to 0.4 μL hot start Taq polymerase. 8 μL of template DNA was added into each PCR reaction tubes containing 12 μL Master mix. The PCR grade water (8 μL) and 8 μL of positive control were considered as negative and positive controls, respectively. Amplications were carried out in master cycler (Eppendorf Co., Germany). Denaturation was performed at 95°C for 3 minutes, followed by 35 cycles (94°C for 30 seconds, 58°C for 40 seconds and 67°C for 40 seconds) and 72°C for 5 minutes. For nested PCR; we transferred 15 μL PCR Master mix 2X, 0.2 μL Taq polymerase and 5 μL of the first PCR products to each PCR reaction tubes. Amplication program was as follow: 95°C for 3 minutes, followed by 35 cycles (94°C for 30 seconds, 56°C for 40 seconds and 67°C for 40 seconds) and 72°C for 5 minutes. The final PCR products were analyzed on 2% agarose gel electrophoresis stained with ethidium bromide. The nested PCR procedure was used to screen 242 samples (121 vaginal and 121 endocervix samples). The first specific primer sets was derived from sequences of the common endogenous plasmid of *C. trachomatis* (15), 517 base pair (bp) amplified products with all known *C. trachomatis* serovars (F = *GGACAAATCGTATCTCGG*, R = *GAAACCAACTCTACGCTG*) (13). The second primer set was F = *ATTGCTTGAGCGTATAAAGG* and R = *TGCTATAATCACGAAATTAC*; the amplified product was 250 bp.

4. Results

We enrolled 121 women aged between 17 - 38 years old with 21% miscarriages in the past, referred to the Medical Centers. There was a significant association between the number of abortions and abnormal vaginal discharge with *C. trachomatis* infection (P = 0.00 and P = 0.001, respectively). In addition, sexual infection among patients with more than 3 intercourses to less than 3 intercourses per week showed significant difference (P < 0.05). The base line data are collected in Table 1.

Table 1. Baseline Data of Cases

Data	Number of Intercourse during a Week		History of Pregnancy		History of Abortion		Vaginal Secretion		Lower Abdominal Pain		Contraceptive Route			
Case	≥ 3	< 3	Yes	No	Yes	No	Yes	No	Yes	No	No [a]	OCP [a]	IUD [a]	Cond [a]
Patients, (%)	65	35	84	16	21	79	32	78	14	86	57	12	18	13

[a] Abbreviations: NO, nothing; OCP, oral contraceptive; IUD, intrauterine device; Cond, condom

The infected samples were identified using nested PCR. Detected rate of *C. trachomatis*, *Mycoplasma genitalium* and *Ureaplasma urealyticum* in the cervixes were 13.25%, 16.5% and 10%, respectively. Frequencies cervical infection with *C. trachomatis*, *M. genitalium* and *U. urealyticum* were 19%, 25% and 25%, respectively (Table 2).

Table 2. Frequency of Infected Sites (by PCR Method)

Infected Site by Bacteria	Endocervix		Vaginal Infection	
	Negative, %	**Positive, %**	**Positive, %**	**Negative, %**
Chlamydia trachomatis	86.75	13.25	19	81
Mycoplasma genitalium	83.5	16.5	25	75
Ureaplasma urealyticum	90	10%	25%	75

5. Discussion

In study of Kucinskiene et al. (2006) the prevalence of *C. trachomatis* in Europe was 4.1 to 25% (16). Sotoodeh Jahromi et al. in 2005, reported that the prevalence of *C. trachomatis* in women with abortion was 25.45% (7). Also, in other studies in different places in Iran, the prevalence of *C. trachomatis* in different cities was 22% (Tehran), 10% (Bandar Abbas) and 6.5% (Shiraz) (17). It seems that these differences are related to sampling and testing methods, sexual behavior, number of partners, hygiene and barrier contraception during intercourse. Meanwhile, adequate specimen has very important role; Welsh and et al. compared the influence of adequate and inadequate sampling on prevalence; 14.2% for adequate and 4.3% for inadequate specimens (18). In our study, abnormal vaginal discharge was the common complaint in women with *C. tracomatis*. The result was the same as the results of Mohammadzadeh et al. study (19). Mucopurulent discharge in Taylor and Haggerty study was 37% (20). *C. trachomatis* was introduced as one of the spontaneous abortion etiological factors (21-23). Wilkowska-Trojniel et al. showed Anti-chlamydial IgA and IgG antibody levels were 7.9% and 21.1% in cases with one miscarriage and were 4.5% and 36.4% in women with two or more miscarriages (2). Elias et al. reported that the rate of cervical chlamydial infection in women with imminent abortion was 26.4% (24). The proportion of abortion in our study was 28.6%. This difference could be related to how sampling and methodology were conducted. Globally, *C. trachomatis* is the most common agent of genital infection (16). Therefore, to prevent abortion, pregnant women (with or without symptom) and high-risk group (male or female) must be screened for *C. trachomatis*. In this study specimens collection were done by swabs but it was not a comfortable method for women patients then we offer urine collection, since it was not invasive. However, it is a simple method and do not need trained personnel but it may be missed other microbial agents such as genital warts and herpes.

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Authors' Contribution

The core idea of this work came from Dr Zahirnia who has also collected the data, cultured as well as performed molecular tests and also wrote the manuscript with the help of Dr Taherpour and Mrs Pourkaveh. Prof Eslami conducted the project as a part of her dissertation. Prof. Goudarzi and Prof Fallah were the advisors of this study. Prof. Taheripanah was the gynecologist and infertility advisor of the present work. Mrs Taheri acted as cultural and advisor and Mrs. Ohadi helped in sample collection.

Financial Disclosure

The authors declare that they have no conflicts of interest.

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