Iranian J Publ Health, 2006, Vol. 35, No. 2, pp.69-75

Comparative Assessment of *Chlamydia trachomatis* Infection in Iranian Women with Cervicitis: A Cross-Sectional Study

*J Zaeimi Yazdi [1], * MR Khorramizadeh [2], N Badami [1], B Kazemi [3], F Aminharati [1], Z Eftekhar [4], A Berahme [2], M Mahmoudi [5]*

[1] *Div. of Microbiology, Dept. of Pathobiology, School of Public Health, Tehran University of Medical Sciences, Iran*
[2] *Div. of Biotechnology, Dept. of Pathobiology, School of Public Health, Tehran University of Medical Sciences, Iran*
[3] *Molecular Biology Center, School of Medicine, Shahid Behehti University of Medical Sciences, Tehran, Iran*
[4] *Mirza Koochakkhan Hospital, Tehran University of Medical Sciences, Iran*
[5] *Dept. of Epidemiology and Biostatistics, School of Public Health, Tehran University of Medical Sciences, Iran*

(Received 11 Jan 2006; revised 22 March 2006; accepted 9 May 2006)

Abstract

Chlamydia trachomatis infections are the most prevalent bacterial sexually transmitted infections (STI) recognized throughout the world. The aim of this study was to determine the prevalence of *Chlamydia trachomatis* among a randomized statistical group of women suffering from cervicitis in Tehran- Iran. During a 12- month- period, Jan 2003 to Jan 2004, 142 endocervical samples were taken from women suffering from cervicitis attending to Mirzakoochakkhan Women Hospital in Tehran, Iran. Direct fluorescent antibody (DFA) and PCR techniques were used to detect *Chlamydia trachomatis* in endocervical samples. Twenty two (15.5%) [95% CI, 9.54-21.4] of 142 samples were diagnosed as *Chlamydia* positive according to PCR results, while DFA diagnosed 20 (14.1%) positive cases [95% CI, 8.37-19.8]. No statistically significant difference was found between two diagnosis methods applied in this study. The prevalence was the highest (25%) among women aged 25 to 29 yr and 35 to 39 yr. The x^2 test showed a significant relationship between positive test result and bearing a history of STI (P= 0). The results of this study showed high prevalence of *C. trachomatis* infection among women suffering from cervicitis and suggested that patients diagnosed with genital *Chlamydia* infection should be referred to the genitourinary medicine clinic for further STI screening and partner notification.

Keywords: *Chlamydia trachomatis*, Cervicitis, DFA, PCR, Iran

Introduction

Chlamydia trachomatis is the most prevalent sexually transmitted disease (STD) in the world, especially in young individuals (1- 4). Among the currently 15 major serovars of *C. trachomatis* (A-C, D-K, L1-L3), serovars D-K are urogenital pathogens (2, 5). In females, *C. trachomatis* may cause arthritis, cervicitis, and pelvic inflammatory disease which may lead to such complications as ectopic pregnancy and tubal factor infertility (4, 6). *C. trachomatis* in the cervix could be transmitted to a neonate during passage through an infected birth canal, resulting in neonatal pneumonia. Vaginal, pharyngeal, and enteric infections in neonates have also been recognized (5, 7). In men however, urogenital serovars cause nongonococcal urethritis and epididymitis. *C. trachomatis* can also induce Reither's syndrome, proctitis, and conjunctivitis in both men and women (5, 8). Infections with *C. trachomatis* have been associated with increased rates of transmission of

***Corresponding Author:** Tel: +98 21 66462268, Fax: +98 21 66462267, E-mail: khoramza@sina.tums.ac.ir

human immunodeficiency virus (HIV) (5). The biggest challenge to the control of chlamydial disease is that as many as 70- 80% of women and up to 50% of men who bear infection do not experience any symptoms (9, 10). This resulted in a large reservoir of unrecognized infected individuals who are capable of transmitting the infection to sexual partners (11).

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 (8). However, the methodological challenges of culturing this organism led to the development of non-culture-based tests, including antigen-detection tests and nucleic acid hybridization. These techniques however, fail to detect substantial proportion of infections. Newer tests are accordingly required to develop that could amplify and detect *C. trachomatis*-specific DNA or RNA sequences, giving rise to more sensitivity than the first generation non-culture-based tests (5, 8).

The aim of this study was to determine the prevalence of *Chlamydia trachomatis* among a randomized statistical group of women suffering from cervicitis in Iran.

Materials and Methods

Study site and population A cross-sectional study on prevalence of *Chlamydia trachomatis* infection among women suffering from cervicitis was conducted between January 2003 and January 2004. One hundred forty two endocervical swabs for diagnosis of *C. trachomtis* were taken from infected women attending to Mirzakoochakkhan Women's Hospital in Tehran, Iran. The study population was women who were examined by a referral gynecologist and diagnosed as cervicitis cases.

Sample collection Briefly, cervical mucus was removed prior to insertion of a cotton swab into the endocervicl canal. The cotton swab was immediately rolled over slides. The slides were air dried, fixed by incubation in methanol, and stored at -20° C until performing DFA (12-14). For PCR detection, a second swab was immersed in 1.5 ml of 2-sucrose phosphate (2SP) transport medium. All 2 SP media were maintained at 4°C during specimen collection and then aliquoted into three microtubes and frozen at -80°C within 4h of collection until DNA extraction (15).

Detection of C. trachomatis by Direct Fluorescent Antibody (DFA) The fixed slides were stained with the IMAGEN ^{TM} *Chlamydia* test (DakoCytomation, Denmark). Briefly, the slides were stained with a fluorescein-conjugted monoclonal antibody (IMAGEN ^{TM} *Chlamydia*) and incubated at 37°C for 15 min. After incubation, they were washed in phosphate-buffer saline, air dried, and examined by an epifluorescence microscope at a magnification of x630 or x1000 for typical apple-green fluorescent elementary bodies (EB). The presence of more than 10 fluorescent EB was considered a positive result (13, 16).

Detection of C. trachomatis by PCR DNA was extracted from endocervical samples using Diatom DNA extraction Kit (IsoGene; Moscow, Russia). To detect the presence of *C. trachomatis* in the cervical DNA samples, a 241-bp fragment of the bacterial endogenous plasmid was amplified (17). The primers used for the *C. trachomatis* plasmid PCR were KL1 (5' TCC GGA GCG AGT TAC GAA GA 3') and KL2 (5' AAT CAA TGC CCG GGA TTG GT 3') (17). PCR was performed in a final volume of 50 µl containing 1.5 mM $MgCl_2$, 200 µM(each) deoxynucleotide triphosphate (dATP, dTTP, dGTP, and dCTP), 50 pM of each primers and 1 U of Taq polymerase. *C. trachomatis* serovar L2 DNA was used as positive control and sample containing distilled water instead of DNA was used as negative control. The amplification was performed with a thermocycler (Techne, Flexigene, TC-412, Cambridge, UK) and started with 10min of denaturation at 94°C; followed by 40 cycles of amplification. Each cycle consisted of denaturation at 94°C for 1 min., annealing at 55°C for 1 min., and extension at 72°C for 1.5 min. The PCR products

were analyzed by 1.5% agarose gel electrophoresis (17).

Statistical analyses: Data analysis was carried out using the Statistical Package for Social Science (SPSS) version 11.5 for Windows. For normally distributed data, means and standard deviations were calculated and compared using the Student's *t* test. Confidence intervals (95%) were reported where appropriate. The x^2 test was applied to compare *Chlamydia* infection and history of STI. To compare age and positive results, logistic regression test was used. Statistical significance was at the 5% level.

Results

The study population was married women aged between 20 and 55 yr (mean age, 32.75 ± 8 and median age: 32). Among the enrolled women, 25% were under 26, 50% were under 32 and 75% were less than 37.2 yr of age, respectively. A history of STI was reported by 38 (26.8%) of study cases. Successful amplification of a 241 bp fragment of *C. trachomatis* plasmid genome was considered as a positive result by PCR (Fig.1). The presence of more than 10 fluorescent EBs was also considered to be a positive result by DFA. Of the 142 endocervical samples tested, 20 (14.1%) were positive for *Chlamydia* in both plasmid-based PCR and DFA (Table 1). For two additional samples there was a disagreement between PCR and DFA methods; i.e., the samples were positive by PCR while had a negative result by DFA. Therefore, overall prevalence of *C. trachomatis* infection was 15.5% (95% CI, 9.54-21.4) and 14.1% (95% CI, 8.37-19.8) by PCR and DFA, respectively. The x^2 test showed no significant statistical difference between PCR and DFA results. Table1 summarizes the rates of prevalence of *C. trachomatis* infection in relation to age according to PCR and DFA results. Prevalence was the highest (25%) among women aged 25 to 29 yr and 35 to 39 yr. No chlamydial infection was seen in women aged 45-49 yr. One of four women in the age group of ≥ 50 yr was *Chlamydia* positive. The results presented in Table 2, shows there was a significant relationship between history of STI and *Chlamydia* infection (OR= 56.66, 95% CI, 12.18 – 263.66).

Table 1: Prevalence of *C. trachomatis* infection in relation to age according to PCR and DFA results

Age groups (yr)	DFA		PCR		Total No. (%)
	Positive No. (%)	Negative No. (%)	Positive No. (%)	Negative No. (%)	
20-24	3 (11.54)	23 (88.46)	4 (15.39)	22(84.61)	26 (18.3)
25-29	7 (25)	21(75)	7 (25)	21(75)	28 (19.72)
30-34	2 (5.9)	32 (94.1)	2 (5.9)	32(94.1)	34 (23.94)
35-39	5 (20.8)	19 (79.2)	6 (25)	18(75)	24 (16.9)
40-44	2 (12.5)	14 (87.5)	2 (12.5)	14(87.5)	16 (11.27)
45-49	0 (0)	10 (100)	0 (0)	10(100)	10 (7.05)
50≤	1 (25)	3 (75)	1 (25)	3(75)	4 (2.82)
Total	20 (14.1)	122 (85.9)	22 (15.5)	120(84.5)	142 (100)

Table 2: Relationship between *Chlamydia* infection and history of STI

	History of STI	
PCR	Positive No. (%)	Negative No. (%)
Positive	20 (90.9)	2 (9.1)
Negative	18(15)	102 (85)
Total	38(26.8)	104(73.2)

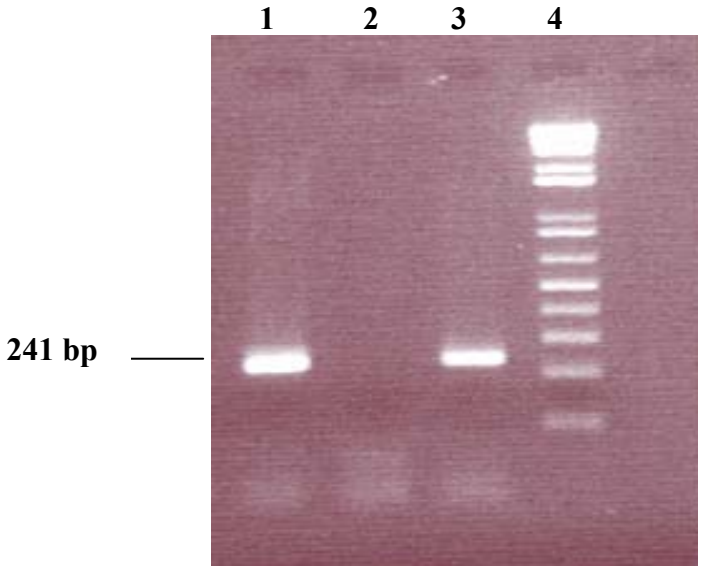

Fig.1: PCR amplification of *C. trachomatis* plasmid DNA
Lane 1: Positive clinical sample, Lane 2: Negative clinical sample,
Lane 3: Positive control, Lane 4: 1 kb plus DNA ladder

Discussion

Chlamydia trachomatis 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 pelvic inflammatory infection (PID), ectopic pregnancy, tubal factor infertility, epididymitis, proctitis and reactive arthritis (5, 8). Chlamydial infections like STI in general, are primarily a women's health care issue since the manifestations and consequences are more damaging to reproductive health in women than in men (5). DFA and PCR techniques are useful and reliable methods for detection of *C. trachomatis* in urogenital samples (8, 11, 12).

This study was performed to determine the prevalence of *C. trachomatis* in women suffering from cervicitis. This is one of the first comparative studies using DFA and PCR assay for detection of *C. trachomatis* in endocervical samples. In the current study, according to the PCR results, we found a prevalence of chlamydial infection of 15.5% among 142 women attending Mirzakoochakkhan Women's Hospital in Tehran. The results of DFA showed a prevalence of 14.1% (Table1). The difference seen between PCR and DFA results might be stemmed from inadequate number of epithelial cells in samples prepared for DFA assay (14), advocating the more sensitivity of PCR technique. There is only one report of prevalence of *C. trachomatis* infections from Iran. In 2002, Fallah et al. showed the prevalence of chlamydial infections as 14.9% among women suffering from cervicitis (2).

The overall 15.5% prevalence of *C. trachomatis* among population under study is comparable to or somewhat higher than that has been reported previously (4, 15, 18).

In a number of studies from Eastern Europe countries, Domeika et al. reported the prevalence of *C. trachomatis* infection in women consulting outpatient gynecological clinics varies between 6 to 25%. We could divide the countries into those bearing low (< 10%), middle (11-20%), and high (>21%) prevalence of *Chlamydia* infections. Of the nine countries where such data were available, four fell within the low prevalence group. These were Slovenia (6%), Hungary (6.3%), Lithuania (8%), and

Poland (10%). Both St. Petersburg region (12.8%) and Bulgaria (16%) belong to the middle prevalence category; while Sverdlovsk region of Russia (22.1%) and Ukraine (25%) fell into the high prevalence group (19-21). Therefore, our results are consistent with the results of St. Petersburg and Bulgaria.

In a multicenter study, the prevalence of *C. trachomatis* infection was determined in several regions in United States by Van Der Pol et al. The site-specific prevalence ranged from 4.8 to 15.1%. The results of our study were in consistent with the prevalence of *Chlamydia* infection in Alabama (15.1%) (18). Gaydos et al. showed the prevalence of *Chlamydia* infection among people under their study as 14.8% which was in agreement with the present results(4).

Welsh et al. observed the prevalence of *Chlamydia* infection as 10.7% among females attending sexually transmitted infection, family planning, and school based clinics (14). In another study, the prevalence was 8% among women attending venereal outpatient's clinics and youth based centers (15). The prevalence of *C. trachomatis* infection among sex workers in Dakar, Senegal, has also been reported using endocervical-swab-based PCR DNA amplification assay. In this study, Strum-Ramirez et al. reported the prevalence of *Chlamydia* infection as 28.5% among sex workers (22). The calculated prevalence of this study results are higher than that of our work, probably due to the selection of very high risk population in the survey performed by Strum-Ramirez et al.

Our results showed that the prevalence of *Chlamydia* infection was higher in women aged 25 to 29 yr and 35 to 39 yr, as compared to the other age groups (Table 1). This finding is in contrast to other studies showing a decline of the prevalence rate after 25 yr of age (4, 5, 10). For example, Norman et al. showed the prevalence of chlamydial infection in the highest risk groups (those aged under 20) in both antenatal and abortion clinics as 12.1% and 12.7%, respectively (23).

However, since the 1990s, Centers for Disease Control (CDC), the Preventive Services Task Force, and several clinic organizations have recommended routine screening for chlamydial infection for all sexually active women aged less than 26 yr (10). In addition to the societal and cultural differences in population investigated, another explanation might be derived from the fact that in countries with religious infrastructures, e.g. Iran, some societal limitations deter people from committing dangerous sexual habits.

The results of this study showed that the rate of *Chlamydia* infection was high among women who had a history of STI (OR= 56.66, 95% CI, 12.18- 263.66), that is consistent with other studies reported previously (2, 4, 5).

In conclusion, our investigation has shown that high prevalence of *C. trachomatis* infection among women suffering from cervicitis in Iran and suggest that patients diagnosed with genital *Chlamydia* infection should be referred to the genitourinary medicine clinic for further STI screening and partner notification.

Acknowledgements

This research has been financially sponsored by the School of Public Health, Tehran University of Medical Sciences, Iran.

References

1. Fioravante FCR, Alvis MDFC, Guimaraes EMDB, Turchi MD, Freitas HAG, Domingos LT (2005). Prevalence of *Chlamydia trachomatis* in asympyomatic Brazilian military conscripts. *Sex Transm Dis*, 32(3): 165-69.
2. Fallah F, Kazemi B, Goudarzi H, Badami N, Doostdar F, Ehteda A et al. (2005). Detection of *Chlamydia trachomatis* from urine specimens by PCR in women with cervicitis. *Iranian J Publ Health*, 34(2): 20-6.

3. Boyadzhyan B, Yashina T, Yataba JH, Patnaik M, Hill CS (2004). Comparison of the APTIMA CT and GC assays with the APTIMA Combo 2 assay, the Abbott LCx assay, and direct fluorescent-antibody and culture assays for detection of *Chlamydia trachomatis* and Neisseria gonorrhoeae. *J Clin Microbiol*, 42(7): 3089-93.
4. Gaydos CA, Theodore M, Dalesio N, Wood BJ, Quinn TC (2004). Comparison of three nucleic acid amplification tests for detection of *Chlamydia trachomatis* in urine specimens. *J Clin Microbiol*, 42(7): 3041-45.
5. Jeffrey FP (2003). Genital chlamydial Infections. *N Engl J Med*, 349:2424-30.
6. Fredlund H, Falk L, Jurstrand M, Unemo M (2004). Molecular genetic methods for diagnosis and characterization of *Chlamydia trachomatis* and *Neisseria gonorrhoeae*: impact on epidemiological surveillance and interventions. *APMIS*, 112: 771-84.
7. Watson EJ, Templeton A, Paavonen J, Russell I, Mardh PA et al. (2002). The accuracy and efficacy of screening tests for *Chlamydia trachomatis*: a systematic review. *J Med Microbiol*, 51:1021-31.
8. Mahony JB, Coombes BK, Chernesky MA (2002). *Chlamydia* and *Chlamydophila*. In: *Manual of Clinical Microbiology*. Eds, Murray PR, et al. 3rd ed. ASM press, Washington, D.C. pp. 991-1004.
9. Honey E, Augood C, Templeton A, Russell I , Paavonen J, Mardh PA (2002). Cost effectiveness of screening for *Chlamydia trachomatis*: a review of published study. *Sex Transm Infect*, 78: 406-12.
10. Shish S, Scholle S (2004). *Chlamydia* screening among sexually active young female enrollees of health plans, United States, 1999-2001. *MMWR Morb Mortal Wkly Rep*, 53(42): 983-85.
11. Black CM (1997). Current methods of laboratory diagnosis of *Chlamydia trachomatis* infections. *Clin Microbiol Rev*, 10(1): 160-84.
12. Raymond J (2005). *Chlamydia* infections: diagnostic procedures. *Arch Pediatr*, 12 suppl 1: S42-4.
13. Dean D, Ferrero D, McCarthy M (1998). Comparison of performance and cost-effectiveness of direct fluorescent-antibody, ligase chain reaction, and PCR assays for verification of chlamydial enzyme immunoassay results for popu-lations with a low to moderate prevalence of *Chlamydia trachomatis* infection. *J Clin Microbiol*, 36(1): 94-9.
14. Welsh LE, Quinn TC, Gaydos CA (1997). Influence of endocervical specimen adequacy on PCR and direct fluorescent-antibody staining for detection of *Chlamydia trachomatis* infections. *J Cli Microbiol*, 35(2): 3078-81.
15. Airell A, Ottoson L, Bygdeman SM, Carlberg H, Lidbrink P, Ruden AK (2000). *Chlamydia trachomatis* PCR (Cobas Amplicor) in women: endocervical specimen transported in a specimen of urine versus endocervical and urethral specimens in 2-SP medium versus urine specimen only. *International Journal of STD & AIDS*, 11: 651-58.
16. Van Der Pol B, Williams JA, Jones RB (1995). Rapid antigen assay for identification of *Chlamydia trachomatis* infection. *J Clin Microbiol*, 33(7): 1920-21.
17. Mahony JB, Luinstra KE, Sellors JW, Chernesky MA (1993). Comparison of plasmid- and chromosomal- based polymerase chain reaction assays for detecting *Chlamydia trachomatis* nucleic acids. *J Clin Microbiol*, 31(7):1753-58.
18. Van Der Pol B, Ferrero DV, Buck-Barrington L, Hook III E, Lenderman C, Quinn T (2001). Multiceter evaluation of the BDProbeTec ET system for detection of *Chlamydia trachomatis* and Neisseria gonorrhoeae in urine specimens, female endocervical swabs, and

male urethral swabs. *J Clin Microbiol*, 39(3); 1008-16.

19. Domeika M, Hellen A, Karabanov K, Chudomirov K, Gruber F, Unzeitig V (2002). *Chlamydia trachomatis* infections in Eastern Europe: legal aspects, epidemiology, diagnosis, and treatment. *Sex Transm Infect*, 78: 115-19.
20. Domeika M, Butylkina R., Hellen A, Spukaite T, Juceviciute V, Morkunaite D (2001). Prevalence of *Chlamydia trachomatis* infections in women attending six women's healthcare units in Kaunas, Lithuania. *Sex Transm Infect*, 77: 459-60.
21. Domeika M, Hellen A, Drulyte O (2000). Genital *Chlamydia trachomatis* infections in Lithuanian women invited for screening via newspaper advertisement: a pilot study. *Sex Transm Infect*, 76: 216.
22. Sturm-Ramirez K, Brumbly H, Diop K, Gueye-Ndiaye A, Sankale J-L, Thior I (2000). Molecular epidemiology of genital *Chlamydia trachomatis* infection in high risk women in Senegal, West Africa. *J Clin Microbiol*, 38(1):138-45.
23. Norman JE, Wu O, Twaddle S, Macmillan S, Macmillan L, and Templeton A (2004). An evaluation of economics and acceptability of screening for *Chlamydia trachomatis* infection, in women attending antenatal, abortion, colposcopy and family planning clinics in Scotland, UK. *BJOG*, 111: 1261-26.