CHLAMYDIA TRACHOMATIS SCREENING BY POLYMERASE CHAIN REACTION AMONG ASYMPTOMATIC TURKISH WOMEN: A PREVALENCE STUDY

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ABSTRACT

Introduction: Chlamydia trachomatis infection is the most common sexually transmitted disease and it represents a major public health problem in women of reproductive age. Chlamydia infection during pregnancy poses major health risks for both pregnant women and their fetuses. Most of the individuals who are infected by C. trachomatis are asymptomatic. This treatable disease can be detected easily and more precisely thanks to new developing diagnostic technologies. Application of screening programs in which highly sensitive methods such as nucleic acid amplification tests (NAAT) could be beneficial in asymptomatic populations.

Materials and methods: Genital Chlamydia trachomatis infection prevalence in asymptomatic Turkish women, who presented for initial prenatal visit or preconception visit at Anadolu Medical Center in western part of Turkey (İstanbul), between 2006 and 2011, was investigated by Polymerase Chain Reaction (PCR). Endocervical specimens collected from 541 women during their vaginal examination were analyzed by COBAS Amplicor Chlamydia trachomatis test. Two hundred fifty nine women were tested during the first visit of their pregnancy, while 282 women were tested during preconception visit. The median age of the women was 30 years, range 16 to 45 years.

Results: Totally 1.85% (n=10) of the patients were positive. Of the positive patients, 20% (n=2) were pregnant, while 80% (n=8) were in preconception period. Genital Chlamydia trachomatis prevalence was determined as 1.85% in asymptomatic Turkish women population.

Conclusion: Chlamydia trachomatis screening in asymptomatic sexually active women should be performed by highly sensitive NAAT tests. Screening of women in reproductive age may reduce major health risks for pregnant women and their newborns; further investigations that will contribute diagnosis of the disease and the determination of national strategies should be performed.

Keywords: Chlamydia trachomatis, PCR, screening, asymptomatic infection, pregnancy.

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Introduction

Today, it is known that Chlamydia trachomatis is the most common sexually transmitted bacterial agent in the world10. Most of C. trachomatis infected individuals are asymptomatic and they can be detected only by carefully designed screening programs10. Pelvic inflammatory disease (PID) occurs in 40% of untreated C. trachomatis infections in women10. Infertility, chronic pelvic pain and ectopic pregnancy are observed in 20%, 18% and 9% of PID cases, respectively10. It was shown that there was a decrease in C. trachomatis infection as well as PID occurrence by effective screening programs15, 6, 7. Complication due to C. trachomatis infection is rare in men; however, detection of the
infection is important in order to prevent spread of infection. C. trachomatis may also lead to serious diseases such as conjunctivitis and trachoma in neonates, and pneumonia and lymphogranuloma venereum in infants. In this study, C. trachomatis was screened by molecular methods in order to prevent gynecological diseases as well as potential complications that can occur during the neonatal period, through mother-infant transmission. Incidence of this microorganism in pregnant women varies significantly between countries. C. trachomatis screening is recommended in United Kingdom (UK) and United States (USA) at least once a year for sexually active young women considering that almost half of infections are asymptomatic.

Cell culture, direct fluorescence antibody test (DFA), ELISA method and nucleic acid amplification (NAAT)-based tests are the most common screening tests. The samples should contain sufficient epithelium cells since C. trachomatis is an obligatory intracellular microorganism. Definitive diagnosis can be performed by microorganism growth in tissue culture. However, this method is time-consuming and is not appropriate for routine use in laboratories. DFA method is rapid, practical and cost-effective, but the specificity and the sensitivity of the test may change due to sample quality and evaluation experiences of physician. NAAT-based tests, that are more sensitive and rapid than cell culture, are the most sensitive tests and recently, they have replaced the cell culture as a diagnostic tool and used for the detection of C. trachomatis in urine as well as endocervical specimens approved by U.S. Food and Drug Administration (FDA). Morre et al. detected the sensitivity and positive predictive value (PPV) of COBAS Amplicor C. trachomatis test for asymptomatic individuals in U.S.A. as 98.8% and 95.4%, respectively.

In this study, we investigated genital C. trachomatis infection prevalence among asymptomatic Turkish women who presented for their initial prenatal or preconception period visit by COBAS Amplicor C. trachomatis test system.

Materials and methods

A total of 562 women without genital complaints from western part of Turkey (Istanbul and Kocaeli) applied to Anadolu Medical Center (AMC) Obstetric clinic during their preconception or first trimester period between the years January 2006-January 2011. C. trachomatis DNA was tested in endocervical specimens collected by physicians during routine gynecological examination with the informed consent of patients. The Local Ethics Committee approved the cross-sectional study (resolution date 25.01.2015).

Statistical analysis

All analyses were performed using Statistical Package for the Social Sciences (SPSS) version 17.0 software (SPSS Inc., Chicago, IL, USA). Results were reported as mean ± standard deviation (SD) or percentages. The Chi-square test and Fisher’s Exact Test was performed to compare the variables. A p-value of less than 0.05 was considered statistically significant. In asymptomatic pregnant women, this test was performed in the first trimester. Sexually transmitted diseases guideline used in U.S.A. was modeled for this purpose.

Specimen collection

Physicians were informed about the endocervical swabs use for sufficient and suitable sample collection according to the manufacturer’s instructions. Endocervical specimens were transferred to the laboratory rapidly in 3ml M4-RT transport fluid (MicroTest, Inc.) at room temperature (18-30 °C).

PCR study

The specimens were stored at -20 °C and tested once a week by COBAS C. trachomatis test system. DNA was isolated from 100 µl suspension of samples according to manufacturer’s instructions (Roche Molecular Systems, U.S.A.). Fifty microliters of the extracted DNA was transferred to the tubes containing “MasterMix” and internal control. These reaction tubes were then loaded on COBAS Amplicor Analyzer (Roche Diagnostics). Twenty one patients were excluded from this study due to PCR inhibitors. The test results were evaluated qualitatively as positive or negative in 541 women’s samples.

Results

Of 541 DNA samples, 10 were found as C. trachomatis positive. The age range of the patients was 16-45, and the mean age was 30±6 years. 259 women were tested during the first trimester, while 282 women were tested during preconception period (Table 1). Of the positive patients, 20% (n=2) were pregnant, whereas 80% (n=8) were in precon-
ception period. The association between pregnancy and C. trachomatis positivity was evaluated by Fisher’s Exact Test. It was determined that there was no statistically significant relation between pregnancy of women in this population and their positivity ($\chi^2=3.172, p=0.069$).

Table 1: Chlamydia trachomatis detection in endocervical specimens of pre-conception and pregnant women by PCR (P-value > 0.05).

*CT: Chlamydia trachomatis

<table>
<thead>
<tr>
<th>Endocervical specimen</th>
<th>CT Positive (n)</th>
<th>CT Negative (n)</th>
<th>Total (n)</th>
<th>CT Positive (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-conception</td>
<td>8</td>
<td>274</td>
<td>282</td>
<td>2.83</td>
</tr>
<tr>
<td>Pregnant</td>
<td>2</td>
<td>257</td>
<td>259</td>
<td>0.77</td>
</tr>
<tr>
<td>Total</td>
<td>10</td>
<td>531</td>
<td>541</td>
<td>1.85</td>
</tr>
</tbody>
</table>

Figure 1: Age distribution chart of all women screened for C. trachomatis has been shown in Figure 1.

Association between age distribution of patients and C. trachomatis positivity has been evaluated by Pearson Chi-Square test and it was determined that there was no statistically significant relation between them ($\chi^2=0.831, p=0.975$). It was identified that 75% of the patients who live in city centers had associate or bachelor’s degree, 45% had their first pregnancy, and 52% used condom and/or withdrawal method for birth control method. It was also determined that these patients were not tested for C. trachomatis before. C. trachomatis positive patients were found as negative two months after appropriate treatment.

Discussion

Screening programs are performed in developed countries in order to detect C. trachomatis infection and to prevent complications in women. Screening is suggested for men at high risk. As a general rule, if a disease is screened, it should be a significant public health problem, its prevalence should be high, there should be a highly sensitive test for screening and the cases should be treated. Genital C. trachomatis infections pose epidemiological, social and economic problems for society because they lead to infertility, PID, endometritis, cervicitis in women, preterm birth in pregnant women, and trachoma, lymphogranuloma venerum and pneumonia in infants.

Approved NAAT tests are considered as optimum tests that can be used in screening in order to diagnose C. trachomatis in endocervical smear, urethral smear and urine specimens. The sensitivity and specificity of these tests are over 90% (13). E.J. Watson et al. reported similar ratios in their meta-analysis and they also revealed that the best technique for C. trachomatis identification was DNA amplification methods (14).

C. trachomatis prevalence was found to be 1.7% among persons aged 14-39 years and 4.7% in young women aged <25 years in USA. According to C. trachomatis surveillance data from the 1999-2008 National Health and Nutrition Examination Surveys (NHANES) chlamydia prevalence varies by age, sex, and race/ethnicity, and screening should be performed annually in women aged <25 years and older women at increased risk (e.g., women who have new or multiple sex partners) (15). In a systematic review in the United Kingdom, C. trachomatis estimated prevalence was 9.0% in high school students, 11.9% in women monitored for family planning clinics, and 26.8% women and men who presented to sexually transmitted disease clinics (16). In European countries, the prevalence ranges between 1.7 to 17% (17, 18). In Australia, C. trachomatis prevalence was 3.14% in 16-25 aged patients who were screened routinely during their first pregnancy. It was also revealed that screening program in this group was cost-effective (19).

In Turkey positivity rates detected in various studies performed by different methods in asymptomatic and symptomatic patients are between 1.1-28.8%. In a study of Ortaelii et al. in 2001, C. trachomatis positivity was detected as 1.9% by Ligase Chain Reaction (LCR) in 569 asymptomatic women who applied to family planning clinic in Istanbul (20). C. trachomatis prevalence was found to be 0.9% in a European Union, Turkish Ministry of Health and Hacettepe University collaboration study (2006-2007), entitled “Investigation of main Sexually Transmitted Infections and HIV in Turkey” in 2060 pregnant women (21).
In this study rapid tests were used for screening C. trachomatis. Eksi et al. detected C. trachomatis prevalence 1.1%, 5.16% and 2.15% respectively in 100 asymptomatic, 170 symptomatic and 100 infertile women by NAAT and DFA methods in the southeast part of Turkey in 2011\(^{(22)}\).

Similar to the other studies performed in Turkey\(^{(21,22,23)}\), in this study the mean age of symptomatic and asymptomatic women who were found to be positive for C. trachomatis was 30±6 years. The reason for the high average age in positive C. trachomatis pregnant or preconception patients than western countries may be explained by being sexually active after marriage due to socio-cultural structure of Turkey, variability in sexual behavior features, and elder age of marriage in high socio-economical level of society. It was determined that general and common demographic features of women who applied to Anadolu Medical Center may offer an insight into the data obtained in this study. It was known that level of maternal education was high (75% bachelor’s or associate degree) among women who applied to our gynecology department. Of the patients in this study, 45% had the first pregnancy. It was also known that 52% of the patients used condom or withdrawal method for birth control, the patients were not tested for C. trachomatis before, and they were “asymptomatic”. The hospital was accepting high socio-economic status of patients in 2006-2011 and offering service to these patients with private health insurance. It should be considered that where the data were obtained from and examined accordingly.

In this sense, data of a part of society who live and behave similar to western can be expected to be close to “Western Europe” countries. However, socially a “Turkey-specific” behavior should be expected from this group in spite of the economic development. The reason for this is the low prevalence of sexually transmitted diseases and relatively monogamous sexual behavior pattern in Turkish population. Hence, the best indicator for this can be HIV (human Immunodeficiency Virus) prevalence in Turkey. This prevalence is very low in our country and it has never reached to a level in Europe (the number of HIV/AIDS cases in our country according to Republic of Turkey Public Health Agency of Health Ministry, Department of Infectious Diseases, and Venereal Diseases Unit was 8238 in June 2014. Of these, 1197 were AIDS and 7041 were HIV infected). Thus, C. trachomatis prevalence is expected to be lower than European countries and USA. It was reported that positive predictive value (PPV) of screening of this bacteria in a single sample by NAAT tests was low in populations with low prevalence. It was suggested that negative patients should be tested by an alternative NAAT test. This depends on C. trachomatis concentration in sample, collection and transport of specimen and patient-specific factors (age, gender, infection phase, previous sexually transmitted disease, symptoms, etc.). Multiple testing should be considered to enhance PPV in future studies in Turkey.

In conclusion, C. trachomatis prevalence was found to be low in our asymptomatic and high socio-economic status of patients than Western culture. Low prevalence is an expected result for a country that has a significant difference in sexual behavior patterns from the western world. Laboratories should utilize methods in which nucleic acids are detected with high sensitivity. This may enable to launch more convenient and less costly screening programs. Large-scaled and controlled studies including common highly sensitive tests on screening of STD such as Chlamydia trachomatis infection in certain patient and age groups are required for National screening strategies.

**References**


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