Assessment of *Chlamydia trachomatis* Prevalence by Cell Culture and Transcription-Mediated Amplification in Symptomatic Women

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Key Words

Chlamydia trachomatis · Mucopurulent discharge ·
Transcription-mediated amplification assay ·
Prevalence, *Chlamydia trachomatis*

Abstract

Objective: The frequency of *Chlamydia trachomatis* in women with mucopurulent discharge was determined by a cell culture technique and a transcription-mediated amplification (TMA) assay in endocervical swab specimens. **Subjects and Methods:** Endocervical swab specimens were obtained from 116 symptomatic patients with genitourinary complaints or abdominal pain. All of the women were married, with an age range of between 19 and 44 (median 29) years. The cell culture assay was used in all specimens. For 75 specimens the TMA assay was also performed. **Results:** Positive cell culture test results were obtained in 6 (5.2%) patients. Among 75 specimens, 2 were positive by both TMA and culture assays, while 1 specimen was positive only by the culture assay. Of those positive for *C. trachomatis*, 5 were in the 19- to 25-year age group, and 1 was in the >25-year age group. All of the patients with positive results were of low socioeconomic status. **Conclusions:** This study revealed a relatively low rate of *C. trachomatis* infections

in symptomatic married women in Turkey. A commercial TMA assay failed to identify all positive patients, in contrast to a 'gold standard' culture assay used in patients having such infections.

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Introduction

Chlamydia trachomatis is now the most prevalent sexually transmitted bacterial pathogen and can cause infections of cervix, urethra, and upper genital tract in women [1]. Cervicitis is the most common presentation in females, with approximately one third of the infected females having concomitant urethritis. Untreated chlamydial infections can ascend the genitourinary tract, resulting in sequelae such as endometritis, salpingitis, and pelvic inflammatory disease, which can lead to infertility and ectopic pregnancies [2]. Additionally, women with chlamydial infections have been shown to have an increased risk of acquiring human immunodeficiency virus infection [3].

The diagnosis of chlamydial infections depended upon cell culture techniques as the 'gold standard' for detection of pathogens in clinical specimens. However, factors such as specimen inadequacy due to collection method, trans-

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Table 1. Age and socioeconomic status of the study population (n = 116)

	<i>C. trachomatis</i> positive		<i>C. trachomatis</i> negative		Total
	n	%	n	%	
Age, years					
19–25	5	7.5	62	92.5	67
25>	1	2.0	48	98.0	49
Socioeconomic status					
Low	6	5.2	87	94.8	93
High	0		23	100.0	23

port time, and storage conditions can negatively influence the sensitivity of culture. Among the most recent diagnostic techniques, nucleic acid amplification tests such as polymerase chain reaction (PCR), ligase chain reaction, or transcription-mediated amplification (TMA) are capable of detecting small numbers of microorganisms, regardless of their viability, in clinical specimens [4].

In this study, the frequency of *C. trachomatis* in women with mucopurulent discharge was determined by culture and TMA assays in endocervical swab specimens.

Subjects and Methods

Patient Population

Endocervical swab specimens were collected from 116 symptomatic women visiting the Outpatient Department of Obstetrics and Gynecology at the Social Security Hospital and Dokuz Eylul University Medical Faculty, Izmir, Turkey, between December 2000 and June 2001. Symptomatic patients had either genitourinary complaints (i.e., vaginal discharge, pelvic pain, dyspareunia) or abdominal pain. The median age of the patients was 29 (range 19–44) years, and all were married. The patients were divided into two socioeconomic groups. The low socioeconomic group was defined as patients with a yearly average income of <USD 1,000.00 and with a low school-leaving age (n = 93). The high socioeconomic group was defined as patients with a yearly average income of USD 15,000–25,000 and with a higher educational status (n = 23). Culture and TMA assays were performed in the Clinical Microbiology Laboratory at Ege University Medical Faculty.

Specimen Collection

The cervical os was cleaned to remove excess mucus and exudate before the collection of the sample. Two endocervical specimens were collected from every patient. One swab was placed into 2 ml 2-sucrose phosphate medium (68.46 g sucrose, 2 g K₂HPO₄, 1 g KH₂PO₄, 100 ml fetal calf serum, 1 liter distilled water), transported to the laboratory, and stored at –70°C until processing for culture.

The other swab (Gen-Probe sample collection kit; Gen-Probe, San Diego, Calif., USA) was used for the TMA assay (n = 75). The swab was submitted to the laboratory at room temperature within 48 h of collection. The sample collection kit tube was vortexed, the swab was removed, and the specimen was stored at –70°C prior to processing.

Culture for *C. trachomatis*

Chlamydial culture was performed using cycloheximide-treated McCoy cells by a shell vial method (cell growth medium: 450 ml Eagle's minimum essential medium, 50 ml fetal calf serum, 10 ml 200 mM L-glutamine, 5 ml 1 M Hepes, 25 ml 8.8% glucose, 5 ml penicillin/streptomycin solution, and 0.5 ml 10 mg/ml gentamicin solution). Briefly, 200 µl of sample was inoculated into shell vials containing McCoy cell monolayers on coverslips. The vials were centrifuged at 1,750 g for 60 min at 25°C. The specimen was aspirated from each vial and replaced with an isolation medium containing cycloheximide: 450 ml Eagle's minimum essential medium, 10 ml fetal calf serum, 10 ml 200 mM L-glutamine, 5 ml 1 M Hepes, 25 ml 8.8% glucose, 5 ml penicillin/streptomycin solution, 0.5 ml 10 mg/ml gentamicin solution, and 2 ml 200 µl/mg cycloheximide solution (Sigma Chemical, St. Louis, Mo., USA). The vials were incubated at 35°C for 48 h. The coverslips were fixed in chilled acetone for 10 min and stained with a fluorescence-labeled monoclonal antibody specific for *C. trachomatis* (MicroTrak *C. trachomatis* culture confirmation test; Syva, Palo Alto, Calif., USA) according to the manufacturer's recommendations. Assays were examined for typical fluorescent inclusions and were considered positive if one or more inclusions were present. Blind passages were not performed [5].

Transcription-Mediated Amplification Assay

The Gen-Probe AMP CT TMA system amplifies a specific chlamydial 23S rRNA target via DNA intermediates. The amplified RNA product (amplicon) is detected by hybridization with a complementary acridinium ester labeled DNA probe. The assay was performed according to the manufacturer's instructions. Negative and positive controls which are supplied in the kit were included in each assay. Statistical analysis was performed using the $\chi^2_{[1]}$ test.

Results

Among the 116 specimens, positive culture results were obtained for 6 (5.2%). Of the 75 paired specimens tested by both culture and TMA assay, 2 were positive by both assays, while 1 was positive only by the culture assay. The reflect light unit values for each positive control were between 950,155 and 2,431,275 and for each negative control between 2,623 and 3,754. The specimen with culture-positive and TMA-negative results was tested twice for confirmation. A first voided urine sample from this patient was tested using a PCR method (Cobas Amplicor CT; Roche Diagnostic Systems, Branchburg, N.J., USA) and found to be positive.

C. trachomatis results and their relationship with age and socioeconomic status are summarized in table 1. Of

those positive for *C. trachomatis*, 5 (7.4%) specimens were in the 19- to 25-year age group and 1 (2%) in the >25-year age group, but the difference between the two age groups did not show statistical significance ($\chi^2_{[1]} = 1.49$, $p > 0.05$). All patients with positive results were of low socioeconomic status.

Discussion

Among the many inflammatory diseases in females, *C. trachomatis* has been implicated as the causative organism in cervicitis, urethritis, acute salpingitis, proctitis, perihepatitis, conjunctivitis, chorioamnionitis, premature labor, and premature rupture of membranes [6]. The incidence of *C. trachomatis* in asymptomatic women in the United States has been reported to be between 2 and 37%. However, the incidence may exceed 40% in some populations [7]. In the studies reported from Turkey using different screening methods in asymptomatic women, prevalences of 8.9 and 9.3% were found [8, 9]. In symptomatic women the prevalence increased to 12–16%, as determined by direct fluorescent antibody test and enzyme immunoassay [10, 11]. The prevalence of *C. trachomatis* infections was 5.2% in the population studied with the cell culture method.

Several studies have identified demographic and behavioral risk factors that are associated with *C. trachomatis* infection, including younger age (particularly <25 years), multiple sex partners, recent change in partner, unmarried status, ethnic group, leaving school at an early age, and genital symptoms or the presence of another sexually transmitted infection [12]. Our study population consisted of married women. No sexually active single women were included in our study because sexual activity and/or admission to a sexually transmitted disease clinic or a gynecologist are not common among unmarried young women because of the traditions and culture in Turkey. Of those patients positive for *C. trachomatis*, 5 were younger than 25 years of age, and the remaining subject was 31 years old. All positive samples were from the low-income group. These results are consistent with those previously reported [see 15], but the difference between the groups was not statistically significant. This may be due to the small size of the study group. The low prevalence observed in this study may be due to the selection of a group that consisted solely of married women.

Traditional cell culture methods have been the 'gold standard' for the diagnosis of chlamydial infections. However, cell culture methods are time-consuming and sub-

ject to technical variability. Nucleic acid amplification tests such as PCR, ligase chain reaction, and TMA assay are gaining wider use. The advantages of nonculture methods include less stringent transport temperature requirements, elimination of the need for maintaining *C. trachomatis* viability during collection and transport, and the capability of detecting small numbers of microorganisms. These assays are highly sensitive, specific, and rapid in the diagnosis of genital *C. trachomatis* infections. On the other hand, it is known that the amplification techniques are sensitive to inhibitors. The commercial PCR assay has its own internal controls, so it is possible to identify clinical specimens containing PCR inhibitors, thus ensuring the integrity of the result [13–16]. In this study, a comparative evaluation of TMA assay and cell culture techniques for the detection of *C. trachomatis* was conducted in 75 samples. Of these samples, 2 were found to be positive using both techniques. The remaining sample was positive only by cell culture. Since a positive result was obtained with PCR of the first voided urine sample from this patient, the negative result of the TMA assay was probably due to the presence of inhibitors in the endocervical specimen [14].

Screening has been shown to reduce the prevalence of chlamydia in women and the incidence of pelvic inflammatory disease. The CDC recommend routine screening of *C. trachomatis* in all sexually active women aged 25 years and younger [17]. In this study, most of the positive samples were from patients in the 19- to 25-year age group, even though the study group consisted of married women. It seems reasonable to suggest that routine screening for *C. trachomatis* should be performed in all sexually active women aged 25 years and younger.

To conclude, the TMA assay is a rapid test for the detection of *C. trachomatis* in endocervical swab specimens which is easy to perform. However, this test did not prove to be as reliable as the 'gold standard' cell culture test. This study provided insufficient data for a comparison of sensitivity and specificity of the assay methods.

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