CHLAMYDIAL INFECTIONS AND THEIR LABORATORY DIAGNOSES
Klamidiyal enfeksiyonlar ve laboratuar tanı yöntemleri

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Abstract

Chlamydia trachomatis (C. trachomatis) infections are the most common bacterial cause of sexually transmitted diseases in the world. In women, these infections often result in such serious reproductive tract complications as pelvic inflammatory disease, infertility, and ectopic pregnancy, and an infected woman can pass the infection on to her newborn during delivery. The pervasiveness of this often asymptomatic disease necessitates that health care providers actively look C. trachomatis infection, especially in young women. C. trachomatis is an obligate intracellular organism therefore tissue culture is the best diagnostic procedure. Antigen detection, the enzyme immunoassay test and nucleic acid hybridization tests remain important challenges. Nucleic acid amplification technologies make non-invasive urine testing cost effective and easily performed in primary care. Historically the diagnosis of C. trachomatis infections has been difficult, but newer chlamydia diagnostic tests have become clinically available in the past decade.

Key Words: *Chlamydia trachomatis, Infection, Polymerase chain reaction, Epidemiology*

Özet

Giriş: *Klamidya trachomatis (C. trachomatis), cinsel yolla bulaşan hastalıklarda izole edilen en sık bakteriyel etkendir. Kadınlarda pelvik enflamatuar hastalık, infertilite veya ektopik gebelik gibi ciddi reprodüktif komplikasyonlara yol açabilmekte ve enfekte bayanlar doğum eylemi sırasında etkeni doğan çocuklarına geçirebilmektedir. Genellikle asemptomatik seyreden bir hastalığın, özellikle genç kadınlardaki sonuçları sağlık çalışanlarının bu etken ile ilgilenmelerine neden olmuştur. C. trachomatis' in bir zorunlu intrasellüler organizma olması nedeniyle doku kültürleri en iyi ayırım yöntemidir. Enzim immunoassay testi ve nükleik asit hibridizasyon testi belli başlı tanı yöntemleri arasındadır. Nükleik asit amplifikasyon teknikleri, invaziv olmayan idrar testlerini birinci basamak sağlık kurumlarında ucuz bir maliyet ile yapılabilir duruma getirmiştir. C. trachomatis infeksiyon tanısı, yeni tekniklerin ortaya çıkması ile her geçen gün daha kolay bir işlem haline gelmektedir.*

Anahtar Kelimeler: *Klamidya trachomatis, Polimeraz zincir reaksiyonu, İnfeksiyon, Epidemiyoloji*

Chlamydia trachomatis (*C. trachomatis*) infections are the most common bacterial cause of sexually transmitted disease (STD) in the world (1). In women, these infections often result in serious reproductive tract complications, such as pelvic inflammatory disease (PID), infertility, and ectopic pregnancy (2). In addition, an infected pregnant women can pass the infection on to her newborn during delivery, resulting in such problems as ophtalmia neonatorum, which appears as conjunctivitis 5 to 12 days after birth. *C. trachomatis* is also a common cause of subacute, afebrile pneumonia in newborns (3,10).

A large number of published studies have examined the prevalence and characteristics of chlamydia infections, mostly among sexually active women attending clinics for family planning, prenatal care, and the diagnosis and treatment of STD (4,12). Regardless of the region of the country or the population density, the prevalence and risk factors

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are similar. The highest prevalence has been reported among sexually active adolescent females 17 years of age and younger in the USA (4,17,18,19). More than 105 of sexually active young women tested in various clinics were found to have chlamydial infections, a level significantly high enough that routine testing for chlamydia is suggested (5,6,7).

A high number of sexual partners and concurrent gonorrhea infections are commonly associated with chlamydial infection. In fact, patients with gonococcal infection are so commonly coinfected with *C trachomatis* that treatment is advised if no diagnostic test for chlamydia will be performed (7,8). While there is no consistent evidence that oral contraceptives raise the risk of chlamydial infection, the data clearly indicate that barrier contraception protects against chlamydial infection (8,13).

Approximately 70% of chlamydial infections and 50% of gonococcal infections in women are asymptomatic. Asymptomatic carriage of chlamydia in men as well as in women may be prolonged, often persisting for months. Little is known about the importance of the sexual transmission of chlamydia, but it appears that chlamydia is more difficult to transmit than gonorrhea (9). In addition, chlamydial infections may facilitate human immunodeficiency virus transmission (10).

Clinical signs

If not adequately treated, women develop PID. Scarring sequelae of PID will cause involuntary infertility in 20% of women, ectopic pregnancy in 9%, and chronic pelvic pain in 18% of women (11).

The endocervix is the most common site infected by *C. trachomatis* however, the urethra and the rectum may also be infected. Most cervical chlamydial infections do not cause sufficient inflammation to result in clinical signs (11,12). When symptoms do occur, they most commonly include vaginal discharge and/or dysuria (12). The presence of green or yellow mucopus on swab from within the cervical os or 10 or more polymorphonuclear leukocytes (PMNs) per oil immersion field of Gram's stained cervical secretions is strongly associated with chlamydial infection and termed as "mucopurulent cervicitis", the female equivalent of urethritis in men (13,22). Some experts reserve the diagnosis of mucoprulent cervicitis for finding of 30 or more PMNs per high-power field on a cervical Gram's stain (13,14) . The propagation of lower genitourinary tract infection to the endometrium and fallopian tubes may cause lower abdominal pain and menstrual abnormalities. The proportion of women with chlamydial infection who develop infection of the upper reproductive tract (including endometritis, salpingitis and pelvic peritonitis) is unknown (14,15).

Pelvic inflammatory disease . The rate at which chlamydial organisms have been recovered from patients with symptoms of PID has varied widely, probably because of differences in the populations being studied and in the methods used to recover the organisms. Investigators from Europe and North America have found a higher proportion of *C trachomatis* than Neisseria gonorrhoeae in women treated for PID (7,15,16,17).

The clinical presentation of symptomatic chlamydial PID is essentially the same as that caused by other organisms, although it appears that symptoms may be milder than those caused by gonococcal PID. The major presenting complaint is lower abdominal pain that is usually constant but may be intermittent. Increased vaginal discharge or fever may or may not be present. Symptoms commonly begin at the time of menstruation (18).

The role of asymptomatic or subclinical chlamydial PID in the development of reproductive problems has assumed greater importance. Colonization of the fallopian tube by *C. trachomatis* has been found in infertile women who have no clinical symptoms of PID and no laparoscopic signs of active pelvic infection. Ectopic pregnancy may result from prior chlamydial tubal damage (19).

Neonatal complication . Infection of neonates with *C. trachomatis* results from perinatal exposure to the mother's infected cervix. The prevalence of *C. trachomatis* infection generally exceeds 5% among pregnant women, regardless of race/ethnicity or socioeconomic status. Initial *C. trachomatis* perinatal infection involves mucous membranes of the eye, oropharynx, urogenital tract, and rectum. Chlamydia is the most frequent identifiable cause of ophthalmia neonatorum and should be considered as the probable etiology for conjunctivitis in all infants who develop conjunctivitis within the first 30 days of life (10,20).

C. trachomatis is also a common cause of subacute, afebrile pneumonia with onset from 1 to 3 months of age. Cough with tachypnea, and hyperinflation and bilateral diffuse infiltrates on a chest roentgenogram is characteristic. Wheezing is rare, and children are typically afebrile. Because variation in this clinical presentation is common, initial treatment and diagnostic tests should encompass *C. trachomatis* for all infants 1 to 3 months of age who have possible pneumonia (4,21).

Laboratory diagnosis

Because chlamydiae are obligate intracellular organisms that infect the columnar epithelium, the objective of good specimen collection should be to obtain columnar epithelial cells from the endocervix or uretra. The diagnosis of chlamydial STDs generally has been difficult and remains a challenge, but newer chlamydia diagnostic tests have been clinically available in the past decade (11,14,22,23).

Cell culture The isolation of *C. trachomatis* in tissue culture was first developed in the 1970s and has been refined over the years. The sensitivity is 70% to 90%, with a specificity of close to 100% (21,22,23).

Tissue culture remains the gold standard, yet its application in clinical settings ranging from university hospital to local family medicine office and public health clinics is limited by a lack of appropriate reference laboratories, technical expertise, funds or recognition of chlamydiae as important STD pathogens. The requirement of at least 3 to 7 days for optimal chlamydial growth diminishes the utility of cell culture. Once the specimen is collected, it must be kept refrigerated for no longer than 24 hours before inoculation onto McCoy cells. The preferred method for detection of chlamydia in tissue culture is with a flourescein-labeled antibody that is specific for *C trachomatis* and reacts with the inclusion body formed inside the cell. Since tissue culture amplifies small numbers of organisms, it is also preferred for specimens in which low numbers of organisms are expected (19,23).

Antigen Detection. New nonculture diagnostic tests, each with their own utility and limitation, were introduced in the 1980s. The direct fluorescent antibody (DFA) test is based on detection of elementary bodies (EB) in patient specimens using a fluorescein - labeled monoclonal antibody that is specific for either the major outer membrane protein of *C. trachomatis* or the lipopolysaccharide (LPS) moiety of the EB. A distinct advantage of DFA is that the quality of the specimen can be assessed. when it is applied to a slide, the direct visualization of epithelial cells in the specimen under fluorescent microscopy indicates an adequate specimen is obtained. Slides can be restored at 4°C for a few months or at -80°C indefinetely. The sensitivity, specificity, and positive predictive values for DFA have been assessed by comparison with culture. In high prevalence populations (>5%), sensitivity varies from 70 % to 90 % depending on the quality of the specimen collected, patients characteristics including age and STD risk factors, and the technical reliability of the laboratory performing cultures. The specificity is from 96% to 99% in the same high prevalence populations (21,24,25). False negative and false positive results can occur but are more of a problem in low prevalence groups (<5%).

The enzyme immunoassay test (EIA). This test employs polyclonal or monoclonal antibodies that detect chlamydial LPS . The antibodies are conjugated with an enzyme that reacts with a substrate to produce a colored product if chlamydiae

are present. A spectrophotometer is required to detect the intensity of the colored product. A major disadvantage of this assay is that the antibodies with the LPS of other bacterial species found in the vagina or urinary tract can produce a false positive result. This is also not species specific for *C. trachomatis.* Most EIA tests contain a blocking antibody that can be used to confirm a positive test. The sensitivities, specificities, and positive predictive values for EIA are similar to those for DFA (14,25,26).

Nucleic acid hybridization. Nucleic acid hybridization (gen-probe) tests use chemiluminescent type DNA probe that is complementary to a sequence of ribosomal RNA in the chlamydial genome of the patient sample. A distinct advantage of this assay is that it is specific for *C. trachomatis* and does not cross react with other bacteriae. Specimens can be stored at room temperature in special transport material and processed within 7 days. The sensitivity and specificity rates are similar to those for DFA and EIA. The availability of nucleic acid amplification technologies may make non-invasive urine testing available for young men and for young women when a gynecologic examination is not otherwise required. Accurate detection of asymptomatic chlamydial disease in a timely, cost effective, and noninvasive manner as well as the development of effective partner treatment strategies remain important challenges. This review provides a clinical update on office based testing for *C. trachomatis,* management and treatment options for the adolescent and young adult population (8,26).

Two additional nucleic acid type assays recently developed were: *ligase chain reaction (LCR) and polymerase chain reaction (PCR).* With these tests, detection is achieved by exponential amplification of a specific DNA target sequence. Studies suggested that LCR and PCR in the urine of both men and women are more sensitive than culture; sensitivities for the nucleic acid tests reach 95% compared with 85% for cultures. A major problem, however, is the interpretation of positive tests in asymptomatic individuals in low prevalence populations; in this situation, the assay may represent residual DNA but non-viable organisms (14, 27).

Leukocyte esterase screening. The leukocyte esterase test (LET) detects enzymes that are released by polymorphonuclear leukocytes. LET only confirms a diagnosis of urethritis; it fails to determine the specific causative agent of urethral inflammation. The test comes in the form of a dipstick on which a purple color is produced when indoxyl carbonate ester is hydrolyzed by leukocyte esterases. At present, LET is recommended only as a screening test for urethritis in adolescent boys. Because further studies are required to assess its usefulness, LET is not recommended for use in older men or in women as a chlamydia screening test (1,4,11,14,25,28).

Serology: Two serologic tests, microimmunofluorescence and complement fixation are available for serological diagnosis of chlamydial infection. Both require a high level of technical expertise, and have little value in the routine clinical care of patients with possible chlamydial genital infections (2,7,24,28,29).

Conclusion

The prevalence and financial impact of *C. trachomatis* infection in Turkey requires that family physicians and gynecologist stay alert for this disease, especially in women, where the sequelae of untreated chlamydial infection are significant.

To reduce the morbidity and subsequent complications associated with *C. trachomatis* infection in Turkey, effective control and prevention strategies must be implemented. Selective screening to detect asymptomatic infection is an essential component of all control programs. Without effective screening programs, women will continue to become infertile and to seek expensive surgery; ectopic pregnancies will occur endangering the mother's life; and newborns will be at increased risk for exposure and will have a greater chance of developing pneumonia and eye infection.

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