

Cervical Chlamydia trachomatis Infection Related Wet Preparation of Vaginal Secretion among Pregnant Women Attending Antenatal Care Clinic

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ABSTRACT

Objective To assess the prevalence of cervical Chlamydia trachomatis infection among the pregnant women and their association with wet preparation of vaginal secretion.

Design Cross-sectional study.

Setting Antenatal care clinic of Songklanagarind Hospital, Faculty of Medicine, Prince of Songkla University.

Subjects The study group was 199 pregnant women who attended antenatal care clinic.

Main outcome measures Chlamydial isolation and wet preparation of vaginal secretion.

Results Cervical C. trachomatis infection was identified in 10 of 199 cases (5%). Nearly two-thirds (6 of 10 cases) of cervical C. trachomatis infection (60%) were less than 25 years old. Most (7 of 10 cases) of cervical C. trachomatis infection (70%) had no abnormal vaginal discharge. There were no herpes simplex virus and gonococca infection among them. The past history of dysuria, pelvic discomfort, pelvic pain, abnormal menstruation, IUD insertion, and STD in their husbands did not show the difference between chlamydiae negative and positive group; including history of preterm delivery, spontaneous abortion, premature rupture of membranes, fetal death among multiparous women. Three findings of microscopic criteria of vaginal discharge were determined : a) PMN leukocyte to epithelium cell ratio more than 1 : 1 ; b) clue cell more than 20% of total vaginal epithelial cells ; c) scanty or no lactobacilli, each and all findings revealed no statistical significant difference between chlamydiae negative and positive group.

Conclusion Cervical *C. trachomatis* infection is usually asymptomatic, and the diagnosis is depended on chlamydial antigen detection more than microscopic finding of vaginal discharge. In low prevalence population, the application of prenatal screening criteria is not well established.

Key words : chlamydial infection, pregnant women, wet preparation

Chlamydia trachomatis is a major cause of mucopurulent cervicitis.⁽¹⁻²⁾ In pregnancy, vertical transmission during vaginal delivery occur in 50-70% of infants, causing neonatal conjunctivitis or chlamydial pneumonia.⁽³⁻⁶⁾ Most studies performed in the United States in the late of 1970s or early 1980s found prevalence rate of chlamydial infection in pregnant women of 7-13%.⁽⁷⁻⁹⁾ Infection occurs most frequently among women aged 15-21 years and declines markedly with advancing age. Epidemiological factors are useful to predict rates of infection among some groups of pregnant women but not to determine the presence of infection among persons.^(3,10)

In Southern Thailand, no preliminary study was reported. Because of this, obstetric centre are hesitant to implement systemic screening of *C. trachomatis*. The objective of this study was to determine the prevalence of cervical chlamydial infection in pregnant women to assess and identify criteria on vaginal secretion for the implementation of a screening programme.

Materials and Methods

Patient population

The study population consisted of 199 pregnant women attending antenatal care clinic, Songklanagarind Hospital between February to May 1995.

Clinical and microscopic evaluation

Each patient was interviewed concerning clinical and obstetric history. Information was

obtained on patient age, marital status, parity, gestational age, history of abnormal vaginal discharge (AVD), history of sexually transmitted diseases (STD), and previous intrauterine device (IUD) contraception practices, and exposure to antibiotics in the previous two weeks. The AVD was defined as any condition that results in : 1) an odorous, offensive discharge ; or 2) creamy or yellow purulent discharge.⁽¹¹⁾ The third condition of hypersecretion or vaginal discharge not associated with pre and post menstruation was omitted because the study population was pregnant women and the physiologic leukorrhea usually appeared.

Antiseptic applied to external genitalia before pelvic examination is forbidden to avoid vaginal contamination. Vaginal secretion can be recovered for examination by picking from the vaginal blade. After the ectocervix was wiped clean with a large cotton swab, endocervical mucus was collected on a white-tipped swab, with care taken to avoid contamination by the vaginal secretions. The endocervical culture for *C. trachomatis* and herpes simplex virus (HSV) was performed with the use of a cotton-tipped plastic swab which was immediately placed in sucrose-phosphate transport medium on wet ice. Subsequent to *C. trachomatis* culturing, another swab was placed in Thayer Martin media for gonococcal culture.

The specimen can be separately put on the same microscopic slide into two places as a drop. The first site add a droplet of saline mixed directly

on the slide, and the latter with 10% KOH. In either case, the preparation is covered with a cover slip. The saline preparation is then briefly warmed for a half of second in alcohol lamp in order to increase the trichomonad motility, and KOH preparation is gently warmed for a few seconds to promote cell lysis and clearly filamentous form of *Candida* species, with care to avoid completely vaporizing the specimen.

The slide should be examined at X 100 and then X 400. If a standard bright field microscope is used, the substage condenser should be racked down or the substage diaphragm closed to increased contrast. Beside of trichomonad motility and filamentous form of *Candida* species, any findings of the following are determined : a) PMN leukocyte to epithelium cell ratio more than 1 : 1 ; b) clue cell more than 20% of total vaginal epithelial cells ; c) scanty or no lactobacilli. Three deviated findings of microscopic criteria were assessed to correlate with cervical *C. trachomatis* infection.

Laboratory methods

Specimen for isolation of *C. trachomatis* were stored in the sucrose-phosphate transport media (0.2M) at -70°C before inoculation onto cycloheximide-treated McCoy cells in shell vial. Growth of *C. trachomatis* was detected with fluorescein-labeled monoclonal antibody (IMAGEN™ Chlamydia ; DAKO Diagnostic Ltd., Denmark) at 48 hrs after incubation. Specimens for isolation of HSV were inoculated into vero cell in shell vial and centrifuged at 1,500 X g 25°C for 1 hr. Growth of HSV was detected by indirect immunofluorescence technique with rabbit anti-HSV type II (DAKOPATIS, Denmark) and fluorescein-conjugated swine anti-rabbit imunoglobulins (DAKOPATIS, Denmark) at 18-24 hrs after inoculation.

Results

The mean age of the 199 pregnant women was 27.78 ± 4.97 years and the range varied from 15-46 years, among these 123 of 199 cases (61.8%) their age were under 30 years old, and among these only 11 of 199 cases (5.5%) were less than 20 years old. Nearly all (194 of 199 cases) were Thai. Only 27 of 199 cases (13.6%) were examined during first trimester of pregnancy, and 85 of 199 cases (42.7%), 87 of 199 cases (43.7%) during second and third trimester respectively. More than half (116 of 199) of them (58.3%) were multiparous. Their occupation were distributed as follows : government employee (25.1%), trained worker (22.6%), business (8.5%), agriculture (7.5%), fisherman (1.0%), and others (35.2%).

Table 1 shows correlation between cervical *C. trachomatis* infection and other factors and findings. Among these, 10 of 199 cases (5.0%) were culture positive for *C. trachomatis* and diagnosed as cervical *C. trachomatis* infection. Nearly two-thirds (6 of 10 cases) of cervical *C. trachomatis* infection (60.0%) were less than 25 years old. Most (7 of 10 cases) of them (70.0%) had no abnormal vaginal discharge.

There were no herpes simplex virus and gonococcal infection among them. The past history of dysuria, pelvic discomfort, pelvic pain, abnormal menstruation, IUD insertion, and STD in their husbands did not show the difference between chlamydiae negative and positive group. In addition, there were no statistical significant difference between history of preterm delivery, spontaneous abortion, premature rupture of membranes, fetal death among parous pregnant women in the chlamydiae negative and positive group.

Few or absent lactobacilli was found in 78 of 189 cases (41.2%) of non-chlamydial group,

Table 1. Correlation between Cervical C. trachomatis infection and other factors or findings

Factors or findings	Chlamydiae negative (189) number (%)	Chlamydiae positive (10) number (%)
obstetric history		
- preterm delivery	12 (6.3%)	1 (10.0%)*
- spontaneous abortion	17 (8.9%)	-
- ectopic pregnancy	3 (1.5%)	-
- premature rupture of membranes	26 (13.7%)	1 (10.0%)*
- fetal death	5 (2.6%)	-
Presence of abnormal vaginal discharge : foul or yellowish		
	45 (23.8%)	3 (30.0%)*
Criteria of microscopic examination		
- few or absent of lactobacilli	78 (41.2%)	3 (30.0%)*
- PMN : epithelial cell ratio 1 : 1	59 (31.2%)	2 (20.0%)*
- clue cell > 20% of total vaginal epithelial cell	21 (11.1%)	2 (20.0%)*
Other diagnosis		
- Normal	121 (64.0%)	9 (90.0%)
- NIL	33 (17.5%)	1 (10.0%)
- VC	28 (14.9%)	-
- VC, TV	1 (0.5%)	-
- TV	3 (1.5%)	-
- Syphilis	3 (1.5%)	-

* No statistical significance, $P > 0.05$

NIL = non infective leukorrhea, VC = vaginal candidiasis, TV = trichomoniasis

and in 3 of 10 cases (30.0%) of cervical C. trachomatis infection. PMN to epithelial cell ratio more than 1 : 1 revealed in 59 of 189 cases (31.2%) of non-chlamydial group, and in 2 of 10 cases (20.0%) of cervical C. trachomatis infection. Lastly, clue cell more than 20% of total vaginal epithelial cell was detected in 21 of 189 cases (11.1%) of non-chlamydial group, and in 2 of 10 cases (20.0%) of cervical C. trachomatis infection. All three findings were found in 9 of 199 cases (4.5%) ; all were chlamydiae negative. Two

findings were found in 42 of 199 cases (21.1%) ; 2 were chlamydiae positive and 40 were chlamydiae negative. One finding was found in 53 of 199 cases (26.6%) ; all were chlamydiae negative. No finding was found in 85 of 199 (44.2%) ; 7 were chlamydiae positive and 88 were chlamydiae negative. Each and all findings of microscopic criteria in vaginal discharge did not show statistical significant difference between chlamydiae negative and positive group. In non chlamydial group (189 cases), the diagnosis were

as follows : 121 cases (64.0%) were normal which there were no abnormal vaginal discharge (AVD) and no other lower genital tract infections (LGTI), 33 cases (17.5%) were non infective leukorrhea (NIL) which had AVD but no other LGTI, 28 cases were vaginal candidiasis (among these, 6 cases were asymptomatic), 1 case was vaginal candidiasis (VC) and trichomoniasis (TV), 3 cases were TV, and 3 cases were secondary syphilis. For cervical *C. trachomatis* infection, there were no other LGTI and secondary syphilis. Among these, 9 cases were normal, and 1 case was NIL.

Discussion

Cervical *C. trachomatis* infection in pregnant women is important not only because most of the infected women are asymptomatic but also the consequences of vertical transmission are rather high and serious. Studies performed in the United States found prevalence rate of chlamydial infection in pregnant women of 7-13%.⁽⁴⁻⁶⁾ In this study, the prevalence of *C. trachomatis* infection (5.0%) is rather low as compared to other studies. Most of them (60.0%) are less than 25 years old, and did not correlate with history of STD in the spouses, and previous IUD insertion. This discrepancy could be related to the sensitivity of the chlamydial culture because the isolation of *C. trachomatis* are considered highly specific but not perfectly sensitive (estimated sensitivity of 82% in pregnant women).⁽¹²⁾

Criteria to performed selective screening of chlamydial infection have been proposed for non pregnant women, but these models have generally been derived from the population with prevalence of more than 9%, and their model involved clinical signs such as cervicitis or swab-induced cervical bleeding.^(13,14) Referred to

chlamydial infection, mucopurulent cervicitis (MPC) was identified in less than 30% of infected women in several studies.^(15,16) The capability to identify the microscopic endocervical mucopus or microscopic findings of vaginal discharge may also vary widely from one physician to the other. In this study we tried to determine the microscopic findings of vaginal discharge, but the result of vaginal examination is not well associated. However, we recommend that all physicians should perform the microscopic study by themselves according to the following advantages ; 1) Immediate examination yields higher sensitivity and specificity of testing. Hanging drop preparation in 1 ml saline is not recommended because it dilutes the concentration of vaginal materials, and also reduce the sensitivity and specificity of testing. 2) Sending the specimen to the laboratory takes a longer time and decreases the possibility to observe the motility of trichomonads. 3) Learning by doing is more superior to correlate the clinical and laboratory findings.

In low prevalence population, the application of pre-screening criteria is attractive. To enhance the predictive value, additional criteria may be applied, the visible mucopus coexisted with microscopic mucopus were applied as clinical diagnosis of MPC in the non-pregnant population. Both visible mucopus coexists with microscopic mucopus (PMN criteria ≥ 10 PMN leukocytes per $\times 1,000$ field when examine at least 5 separate area or the presence of ≥ 30 PMN leukocytes per $\times 1,000$ field when examine at least one separate area on a stained-smear)^(1,2) may be applied for *C. trachomatis* screening among pregnant women. In addition, assuming a sensitivity of 81% and a specificity of 97% with an ELISA test,⁽¹⁷⁾ the positive predictive value of screening should be improved.

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