J Obstet Gynecol India Vol. 56, No. 6 : November/December 2006 Pg 511-513

ORIGINAL ARTICLE

The Journal of Obstetrics and Gynecology of India

Prevalence of genital Chlamydia trachomatis by first void urine polymerase chain reaction test in women attending out patient clinic

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OBJECTIVE(S) : To study the prevalence of genital Chlamydia trachomatis by first void urine polymer chain reaction (PCR) test.

METHOD(S) : This case-control study was performed on 100 (50 symptomatic, 50 asymptomatic) randomly selected women attending Gynecological and Family Planning outpatient departments. PCR for Chlamydia trachomatis was done on first void urine by in house PCR test by KL-1 and KL-2 plasmid primers. Wet mount and gram stained vaginal smear were studied for presence of Trichomonas vaginalis, Candida spp and bacterial vaginosis by Nugent's criteria. Statistical analysis was done by chi square test.

RESULTS: Genital Chlamydia trachomatis infection was present in 14% of symptomatic and 4% of asymptomatic women (P=0.081). Lower abdominal pain and cervical erosion were found to be significantly associated with presence of Chlamydia trachomatis.

CONCLUSION(S): Universal screening for genital Chlamydia trachomatis should be done in reproductive age group.

Key words : Chlamydia trachomatis, first void urine, polymerase chain reaction

Introduction

Chlamydia trachomatis (CT) infections are among the most common sexually transmitted infections with an estimated 89 million new cases occurring worldwide each year [2]. This infection is a spectrum of diseases spanning asymptomatic illnesses, urethritis, cervicitis, and pelvic inflammatory disease [2].

About 70-90% of endocervical infections in women caused by CT are asymptomatic and these infections may persist for months and even years [1]. They are of considerable importance epidemiologically as usually they are neither detected nor treated and hence represent an important reservoir for chlamydial infections in women. In addition asymptomatically infected women may be at risk of serious reproductive sequelae like ectopic pregnancy, infertility, and pelvic inflammatory disease [1,2]. Chronic chlamydial infection along with human papilloma virus is also associated with cervical intraepithelial neoplasia [3].

Culture analysis of endocervical swab samples has been considered the diagnostic gold standard for detection of cervical chlamydial infection in women, but factors like sample collection, transportation time, storage of sample, and toxicity of swab can decrease the sensitivity [4]. These problems have made studies of Chlamydia prevalence difficult and some times unreliable. Therefore, other methods have been developed in recent years. Tests using direct immunofluorescence, enzyme immunoassays, and DNA probe technics have been described. Generally, the specificity of these technics is satisfactory but they lack good sensitivity compared with cell culture [4]. The introduction of assays based on nucleic acid amplification technics has subsequently increased the sensitivity of detecting chlamydial infections. They have made possible, detection of CT in urine specimen for both men and women [5].

Paper received on 06/07/2005 ; accepted on 09/02/2006

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The use of nucleic acid amplification technology to detect infected males and females by examination of first void urine is a newly recognized noninvasive screening method that can be easily employed for population based screening. Urine based screening also affords access to large populations particularly asymptomatic men and women who may not visit health clinics [6]. This study was undertaken to find the prevalence of CT infection in symptomatic as well as asymptomatic women and to assess the relationship of CT with other vaginal infections.

Methods

A total of 100 women were randomly selected from patients attending Gynecological and Family Planning out patient departments from 1st July, 2003 to 31st August, 2004. Women coming with pathological vaginal discharge or perineal itching or lower abdominal pain (n=50) formed the study group while women with none of the above complaints suggestive of genital tract infection (n=50) formed the control group. This group included women coming for contraceptive advice, laparoscopic tubal ligation, copper-T check, and follow- up after abortion or vaginal delivery or cesarean section. Informed consent was obtained from all the women. Women were excluded from the study if they were not sexually active, if their age was < 15 or > 45 years, if they were pregnant or menstruating, had antibiotics in previous 10 days, and were having cancerous or precancerous lesion of the cervix detected by clinical examination and / or Pap smear. Demographic details and a detailed personal and sexual history were recorded. Thorough general, systemic, abdominal, speculum, and vaginal examinations were performed, and lower abdominal tenderness, cervical erosion, adnexal mass, and tenderness in the fornices were noted, if present. Color, amount and pH of the vaginal discharge (by pH paper) and the presence of foul smell after adding 10% KOH to it were also noted.

Specimen collection

All women were asked to collect in a sterile plastic bottle, 30 mL of the first void urine (first part of urine passed any time during the day), for polymerase chain reaction (PCR) for CT. Vaginal discharge was taken from posterior vaginal fornix by a sterile cotton swab. It was used to form a thin smear on a clean glass slide for Gram's staining. In study cases a wet mount smear was also made on a separate slide. Besides 3 mL of blood was collected in sterile syringe, and serum separated and stored at 2-8^{0}C for tests for HIV and VDRL.

PCR for Chlamydia trachomatis was done in the department of microbiology by using in house PCR method with KL-1 and KL-2 plasmid primers [7]. Wet mount smear was examined for presence of Trichomonas vaginalis (TV) by its rotatory movement and for pseudohyphae of Candida spp.

Gram stained vaginal smears were examined for clue cells (squamous epithelial cells covered with Gardenerella vaginalis), budding yeast, and pseudohyphae of Candida spp. Diagnosis of bacterial vaginosis (BV) was made by Nugent's scoring as well as by Amsel's criteria. Tests for HIV and VDRL were performed in study cases only after taking informed consent. HIV was tested by dot immunoassay by Combaids-RS test kit as per manufaturer's instructions. Qualitative and quantitative analysis for VDRL was done by slide flocculation test.

Chi square test was used to compare the prevalence of CT and bacterial vaginosis in study cases and controls and the relationship of various risk factors for CT and other genital tract infections. The significance of results were verified by Fisher's exact probability test and chi square test with Yate's corrections.

Results

The overall prevalence of genital CT was found to be 9% (9/100). Its prevalence in symptomatic population was 14% (7/50) and in asymptomatic population 4% (2/50) (P=0.081). Presence of lower abdominal pain and cervical erosion were significantly associated with Chlamydia positivity as shown in Table 1 (P=0.01 and 0.034 respectively).

Table 1. Association of presenting symptoms and signs with Chlamydia trachomatis PCR positivity.

Presenting symptoms	PCR positive	PCR negative	P-value
Lower abdominal pain (n=41)	7 (17.07)	34 (82.93)	0.01 Significant
Urinary complaints (n=20)	3 (15)	17 (85)	0.295
Perineal itching (n=16)	2 (12.5)	14 (87.5)	0.594 Significant
Vulval inflammation (n=2)	0 (0)	2(100)	0.653
Cervical erosion (n=26)	5 (19.23)	21 (80.77)	0.034 Significant
Lower abdominal tenderness (n=26)	4 (15.38)	22 (84.62)	0.359
Adnexal tenderness (n=32)	5 (15.62)	27 (84.38)	0.112

Figures in brackets represent percentages.

Among all microorganisms tested, BV (by Nugent's criteria) was most common, with a prevalence of 30% among study cases and 8% among controls. A higher score by Nugent's grading is associated with a higher frequency of Amsel's positivity.

The association of CT with other vaginal microorganisms was not statistically significant. 10.53% Nugent's positive for BV women were found to be PCR positive for CT. Pseudohyphae of Candida spp were identified in only one CT positive case and Trichomonas was not present in any of the CT positive cases. Tests for HIV and VDRL were negative in all the cases. Vaginal pH >4.5 was significantly associated with BV (P=0.014) but not with any other organism like TV (P=0.096), Candida (P=0.731) and CT (P=0.64)

Discussion

Screening of women for genital Chlamydia trachomatis infection has been recommended by different organizations including Institute of Medicine as a cost effective program which could prevent the high cost of managing sequelae of untreated infection [8]. A reliable estimate of prevalence of genital chlamydial infection in India is not available.

Singh et al [9] detected CT infection by PCR on endocervical specimen in 43.1% symptomatic women attending a gynecology clinic in a city hospital. Mittal et al [10] also found a prevalence rate of 41% in patients presenting with vaginal discharge (as against 14% in our study) and 36% in infertile women by using Papanicolaou and Giemsa cytology. Jensen et al [11] also found the overall prevalence of CT infection to be 11.5% in a prospective study in a STD clinic by using PCR and EIA test on urine sample.

In a comparative study reported by Paukku et al [12] overall prevalence of CT infection was 5.6% among asymptomatic women, using FVU-PCR and endocervical swab antigen testing. This is comparable to our 4% incidence.

Among the high risk factors associated with chlamydial infection lower abdominal pain and cervical erosion were found to be significant (P=0.01 and P=0.034). According to Sorbi and O'Shaughnessy [13] the probability of finding CT infection was 53% in those with lower abdominal pain and 33% in those with dysuria and frequency. Hanna et al [14] have shown that CT, TV. Mycoplasma hominis and Ureoplasma urealyticum were found 2-3 times more often in women with higher vaginal pH. But we have found a significant association of higher vaginal pH only with BV (P=0.014).

Bontis et al [15] have also reported that chlamydial infection is related to visible cervical ectropion presumably because larger area of susceptible tissue is exposed to infection.

Conclusion

We found no statistical difference between prevalence of CT in symptomatic and asymptomatic women. Hence we recommend universal screening for CT in women of reproductive age group. Urinary PCR seems to be a simple, inexpensive, noninvasive, and reliable test for CT screening.

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