Original Article

Prevalence of Positive PCR Assay for *Neisseria gonorrhoeae* and *Chlamydia trachomatis* among Asymptomatic Thai Males Attending STD Clinic at Thailand's Tertiary Referral Center

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Background: Asymptomatic *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (GC) infections continue to be a global public health concern.

Objective: To investigate the prevalence of, and the factors associated with positive urine polymerase chain reaction (PCR) assay for CT and/or GC infection among asymptomatic patients.

Materials and Methods: The present study was a cross-sectional study conducted with positive urine polymerase chain reaction (PCR) assay between August 2015 and September 2016.

Results: One hundred eight patients were recruited. Mean age was 41.2 years. The overall prevalence of positive urine PCR for CT and/or GC in asymptomatic patients was 9.3%. Eight (7.4%) were positive for CT, three (2.8%) were positive for GC, and one (0.9%) patient tested positive for both organisms. In multivariate analysis, younger than 40 years (adjusted OR 14.5, 95% CI 1.4 to 166.7; p=0.027), education less than bachelor's degree (adjusted OR 6.1, 95% CI 1.0 to 35.7; p=0.045), and unemployment (adjusted OR 18.5, 95% CI 1.3 to 250.0; p=0.034) were found to be independent predictors of positive urine PCR for CT and/or GC infection.

Conclusion: The overall prevalence of positive urine PCR for CT and/or GC infection in asymptomatic patients was 9.3%. Younger than 40 years, education less than bachelor's degree, and unemployed status were found to be independent predictors of CT and/or GC infection.

Keywords: Positive PCR, *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, Asymptomatic males, STD

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Sexually transmitted disease (STD) is a major health problem worldwide. The global prevalence of asymptomatic *Chlamydia trachomatis* (CT) infection and asymptomatic *Neisseria gonorrhoeae* (GC) infection in the general population was reported to range from 1% to 12%[1-5], and 0% to 1.5%[5,6], respectively. Asymptomatic males are a potential public health risk, because they are at increased risk for spreading undiagnosed STD infections to their sexual partners. Undiagnosed and untreated STD infections increase disease transmission and make STD containment difficult. The identification of risk factors associated with asymptomatic STD infection would help to decrease disease transmission and improve public health.

Screening test plays an important role in STD detection. Urine examination for CT and GC infection by polymerase chain reaction (PCR) assay was shown to be a highly effective screening tool due to its high sensitivity and specificity[7-10]. Another study found PCR to be an effective screening method in a population with an STD prevalence of as low as

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4%[11]. However, PCR screening for CT and GC infection is not routine in many developing countries due to the relatively high cost of the test. As such, PCR-based screening is often limited to high-risk populations in these countries, especially in asymptomatic males attending an STD clinic that describe high-risk sexual behaviors, history of STD, and/or current infection.

Accordingly, the aim of the present study was to investigate the prevalence of, and the factors associated with positive urine PCR assay for CT and/or GC infection among asymptomatic males attending the STD clinic at Thailand's tertiary referral hospital.

Materials and Methods

The protocol for the present study was approved by the Siriraj Institutional Review Board (SIRB), Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand (SIRB approval no. Si 596/2014). This cross-sectional study was conducted in male patients aged 18 years or older who attended the STD clinic at Siriraj Hospital between August 2015 and September 2016. Some patients had signs and symptoms of other venereal diseases. Some patients with history of unsafe sex or contact to patients with STDs visited the clinic to check up even though they had no signs and symptoms. The patients with any clinical manifestation to suspected urethritis within the previous three months, including urethral discharge, dysuria, urinary incontinence, or frequent urination symptoms, were excluded. Siriraj Hospital is Thailand's university-based national tertiary referral center. Demographic, behavioral, and clinical data, including age, education, occupation, number of sexual partners, sexual orientation, history of contact with sex workers, frequency of condom use, HIV infection status, and history of STD were collected, recorded, and analyzed. History of multiple partners was defined as having more than one sexual partner at the same time. To investigate for CT and GC infection, first-void urine specimens were collected and tested using a CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad Laboratories, Hercules, CA, USA). Urinalysis and intraurethral swab culture were also performed to evaluate for GC. The specimen collected from oropharyngeal swab and rectal swab have not been recommended in the authors' molecular microbiology laboratory yet. Written informed consents were obtained from all participants prior to their inclusion in the study. Patients who had urethral symptoms or currently received antibiotic medication for any reason were excluded from the study.

Table 1. Demographic and clinical characteristics of 108 asymptomatic males attending the STD clinic at Siriraj Hospital

Characteristics	n (%)
Age (years)	
Mean±SD	41.2±15.6
Minimum, maximum	18, 77
Education	
No education	1 (0.9)
Primary	6 (5.6)
Secondary	23 (21.3)
Vocational	5 (4.6)
Bachelor	69 (63.9)
Master	4 (3.7)
Occupation	
Government employee	52 (48.1)
Business employee	36 (33.3)
Student	11 (10.2)
Unemployed	5 (4.6)
Business owner	4 (3.7)
Risk behavior information	
Multiple partners	52 (48.1)
Homosexuality	39 (36.1)
History of contact with sex workers	19 (17.6)
Condom use	
Always	40 (37.0)
Sometimes	34 (31.5)
Never	34 (31.5)
HIV-positive	48 (44.4)
History of STDs*	
Genital or anal warts	46 (42.6)
Syphilis	30 (27.8)
Herpes genitalis	11 (10.2)
Gonorrhea urethritis	9 (8.3)
Non-gonococcal urethritis	4 (3.7)
Genital candidiasis	2 (1.9)
Molluscum contagiosum	2 (1.9)
Chancroid	1 (0.9)

STD=sexual transmitted disease; SD=standard deviation; HIV=human immunodeficiency virus

* Some patients had more than one previous episodes of STD

Table 2. Demographic, behavioral, and clinical data of 10 asymptomatic males who had positive urine PCR assay for GC and/or CT

Case	Age	Education	Occupation	History of STDs	Multiple partners	Homo-sexuality	Contact with sex workers	Condom use	Urine PCR assay		Bacterial culture	Urinalysis (WBCs per high-power field)	HIV infection
									GC	CT			
1	17	Primary	Unemployed	Syphilis	Yes	No	No	Never	Negative	Positive	*S. aureus* (MSSA)	3 to 5	No
2	19	Secondary	Business employee	Genital warts	Yes	No	No	Never	Negative	Positive	No growth	0 to 1	No
3	25	Secondary	Business employee	Chancroid	No	No	No	Never	Negative	Positive	No growth	5 to 10	No
4	33	Bachelor	Business employee	Genital warts	No	No	No	Never	Negative	Positive	No growth	20 to 30	No
5	37	Bachelor	Government employee	Genital warts	No	No	No	Sometimes	Negative	Positive	No growth	0 to 1	No
6	60	Secondary	Government employee	No history	Yes	Yes	Yes	Sometimes	Negative	Positive	No growth	0 to 1	Yes
7	77	No education	Unemployed	Genital warts	Yes	No	Yes	Sometimes	Negative	Positive	No growth	0 to 1	No
8	18	Vocational	Student	Anal warts	No	Yes	No	Always	Positive	Negative	GC	0 to 1	Yes
9	35	Bachelor	Government employee	Genital warts	No	No	No	Sometimes	Positive	Negative	No growth	0 to 1	No
10	22	Bachelor	Unemployed	Anal warts	No	Yes	No	Sometimes	Positive	Positive	No growth	0 to 1	Yes

STD=sexual transmitted disease; PCR=polymerase chain reaction; GC=*Neisseria gonorrhoeae*; CT=*Chlamydia trachomatis*; HIV=human immunodeficiency virus; WBCs=white blood cells; MSSA=methicillin-sensitive *Staphylococcus aureus*

Statistical analysis

Data analyses were performed using SPSS Statistics version 18.0 (SPSS Inc., Chicago, IL, USA). Categorical data were presented as number and percentage, and continuous data were presented as mean ± standard deviation (SD). Association between patients with positive PCR results for CT and/or GC and demographic data or high-risk behavior data were analyzed using chi-squared test or Fisher's exact test, as appropriate. Univariate analysis was performed to individually evaluate the predictive significance of each factor. All risk factors with a p-value of less than 0.2 in univariate analysis were included in multiple logistic regression analysis. Findings from univariate and multivariate analysis were presented as odds ratio (OR) with 95% confidence interval (CI) and adjusted OR with 95% CI, respectively.

Results

One hundred and eight asymptomatic male patients were recruited and enrolled. No one had previous history of dysuria, urethral discharge, or urethritis within one year. Demographic and clinical characteristics are shown in Table 1. Mean±SD age was 41.2±15.6 years (range: 18 to 77). Most subjects had a bachelor's degree (63.9%) and/or worked as a government employee (48.1%). The overall prevalence of positive urine PCR for CT and/or GC infection among males who attended the present study STD clinic was 9.3%. Eight of 108 patients (7.4%) were positive for CT, three of 108 patients (2.8%) were positive for GC, and one patient (0.9%) tested positive for both organisms. Demographic, behavioral, and clinical data of 10 asymptomatic males who had positive urine PCR assay for GC and/or CT are shown in Table 2.

In univariate analysis, unemployed status was the only factor found to be significantly associated with positive urine PCR result for CT and/or GC infection (OR 20.6, 95% CI 2.9 to 144.1; p=0.005) (Table 3). In multivariate analysis, younger than 40 years (adjusted OR 14.5, 95% CI 1.4 to 166.7; p=0.027), education less than bachelor's degree (adjusted OR 6.1, 95% CI 1.0 to 35.7; p=0.045), and unemployment (adjusted OR 18.5, 95% CI 1.3 to 250.0; p=0.034) were found to be independent predictors of positive urine PCR for CT and/or GC infection. Inconsistent condom use (condom used sometimes or never) showed a borderline correlation with positive PCR result, and that relationship failed to achieve statistical significance (p=0.069) (Table 4).

All patients with positive urine PCR for CT were

Table 3. Univariate analysis for factors associated with positive urine PCR assay for CT and/or GC in 108 asymptomatic males

Factors	Urine PCR for CT and/or GC, n (%)		Odds ratio (95% CI)	p-value
	Positive (n=10)	Negative (n=98)		
Age <40 years	8 (80.0)	49 (50.0)	4.0 (0.8 to 19.8)	0.098
Education: less than bachelor's degree	6 (60.0)	29 (29.6)	3.6 (0.9 to 13.6)	0.074
Unemployed	3 (30.0)	2 (2.0)	20.6 (2.9 to 144.1)	0.005*
Risk behavior information				
Multiple partners	4 (40.0)	48 (49.0)	0.7 (0.2 to 2.6)	0.744
Homosexuality	3 (30.0)	36 (36.7)	0.7 (0.2 to 3.0)	1.000
History of contact with sex worker	2 (20.0)	17 (17.3)	1.2 (0.2 to 6.1)	1.000
Inconsistent condom use	9 (90.0)	59 (60.2)	5.9 (0.7 to 48.8)	0.088
Tattoo	1 (10.0)	9 (9.2)	1.1 (0.1 to 9.7)	1.000
Intercourse within 30 days	4 (40.0)	60 (61.2)	0.4 (0.1 to 1.6)	0.311
HIV infection	3 (30.0)	45 (45.9)	0.5 (0.1 to 2.1)	0.507
History of STDs				
Genital or anal warts	7 (70.0)	39 (39.8)	3.5 (0.9 to 14.5)	0.094
Syphilis	1 (10.0)	29 (29.6)	0.3 (0.03 to 2.2)	0.278
Herpes genitalis	0 (0.0)	11 (11.2)	0.0 (0 to 0)	0.594
Gonorrhea urethritis	0 (0.0)	9 (9.2)	0.0 (0 to 0)	1.000
Non-gonococcal urethritis	0 (0.0)	4 (4.1)	0.0 (0 to 0)	1.000
Genital candidiasis	0 (0.0)	2 (2.0)	0.0 (0 to 0)	1.000
Molluscum contagiosum	0 (0.0)	2 (2.0)	0.0 (0 to 0)	1.000
Chancroid	1 (10.0)	0 (0.0)	0.0 (0 to 0)	0.093

PCR=polymerase chain reaction; GC=*Neisseria gonorrhoeae*; CT=*Chlamydia trachomatis*; STD=sexual transmitted disease; CI=confidence interval; HIV=human immunodeficiency virus

* p<0.05 indicates statistical significance

Table 4. Multivariate analysis for factors associated with positive urine PCR assay for CT and/or GC infection in 108 asymptomatic males

Factors	Urine PCR for CT and/or GC, n (%)		Adjusted odds ratio (95% CI)	p-value
	Positive (n=10)	Negative (n=98)		
Age <40 years	8 (80.0)	49 (50.0)	14.5 (1.4 to 166.7)	0.027*
Education: less than bachelor's degree	6 (60.0)	29 (29.6)	6.1 (1.0 to 35.7)	0.045*
Unemployed	3 (30.0)	2 (2.0)	18.5 (1.3 to 250.0)	0.034*
Inconsistent condom use	9 (90.0)	59 (60.2)	8.3 (0.8 to 83.3)	0.069

PCR=polymerase chain reaction; GC=*Neisseria gonorrhoeae*; CT=*Chlamydia trachomatis*; STD=sexual transmitted disease; CI=confidence interval

* p<0.05 indicates statistical significance

treated with a single oral 1-gram dose of azithromycin. All eight of those patients had a negative urine PCR result within six to 14 days. The three patients that tested positive for GC were treated with a single IM 250 mg dose of ceftriaxone and a single oral 1-gram dose of azithromycin. The three GC patients had a

negative urine PCR result within 28 days. The one with both GC and CT infection received with a single IM 250 mg dose of ceftriaxone and a single oral 1-gram dose of azithromycin and cleared the infection within one month.

Discussion

Previous studies in asymptomatic males that attended STD clinics in the United States reported that CT infection rates ranged from 3.8% to 17%[9,12-14]. Another study from the U.S. reported asymptomatic GC infection in 8% of patients[12]. Studies from Asia and Europe in asymptomatic males who attended STD clinics reported incidence rates of CT infection that ranged from 2.6% to 14%[15-17]. The present study found positive urine PCR result for CT and GC of 7.4% and 2.8%, respectively, which was similar to the previous studies. A survey-based study conducted in young Thai men revealed a prevalence of asymptomatic CT and GC infection from PCR test of first-void urine of 4% and 0.2%, respectively[18]. The present study found a higher prevalence in asymptomatic males attending STD clinic. This may be due to screening of high-risk patients.

U.S. Centers for Disease Control and Prevention (CDC) guideline 2015 recommends screening for CT in men should be considered in high prevalence settings, such as adolescent clinics, correctional facilities, and STD clinics. Male homosexuals were also recommended for CT and GC screening[19]. However, the authors did not find evidence of homosexuality associating with positive PCR for CT or GC in the present study.

In the present study, independent predictors of positive CT/GC screening test result from multivariate analysis were age of less than 40 years, education of less than bachelor's degree, and unemployed status. Other studies also reported that CT infection was more common in younger men[15,20]. Where the present study found a higher prevalence of CT or GC in men younger than 40 years of age, other studies reported that younger than 30 years was significantly associated with CT infection[13,17]. Similar to the present study, an education level lower than bachelor's degree was also previously reported to be significantly associated with CT infection[15].

Other factors that were previously reported to be associated with CT infection were inconsistent condom use[2-4,17], having multiple sexual partners[1,2,15], and having sexual intercourse within 30 days[14]. The present study found a borderline association between positive PCR result and inconsistent condom use. However, the study has insufficient power to establish significant association due to small study population. In contrast to previous reports, the authors did not find significant association between multiple sexual partners and positive PCR result for CT or GC[3,21].

Pelvic inflammatory disease (PID) can develop in women as a result of CT or GC infection. Since PID-related conditions, such as ectopic pregnancy, infertility, chronic pelvic pain, and neonatal infection have been associated with significant morbidity and mortality, screening in young and high-risk women have been highly recommended[19,22]. Screening in men was also found to be cost-effective, based on reduction of PID-related complications in their female partners[23].

The present study has some limitations. First, the study population was relatively small. This may have limited the ability to identify all significant associations between factors and positive PCR finding. Second, the present study population was recruited from one center located in a major urban metropolis. Third, the authors' center is Thailand's national tertiary referral center, which means the referred patients are complicated and have intransigent conditions. As such, it is possible that the findings may not be generalized to the patients with the same condition in other regions of Thailand. Fourth, the present study lack the data on previous history of medical treatment for any conditions especially for dysuria or urethral discharge or GC within the last two years. The last limitation is that the collected specimens for diagnosis of CT or GC infection were only urine. The oropharyngeal and rectal swabs were not performed.

In conclusion, the overall prevalence of positive urine PCR for CT or GC infection among males attending the STD clinic was 9.3%. In multivariate analysis, younger than 40 years, education of less than bachelor's degree, and unemployed status were found to be independent predictors of positive urine PCR for CT or GC infection.

What is already known on this topic?

Previous studies reported chlamydia and gonorrhea infections in asymptomatic males who attended STD clinics. There were many risk factors associated with those infections. However, there has been limited study about this topic in Thailand.

What this study adds?

The overall prevalence of positive urine PCR

for CT or GC infection among males attending the present STD clinic was 9.3%. Younger than 40 years, education of less than a bachelor's degree, and unemployed status were found to be independent predictors of positive urine PCR for CT or GC infection. These results may lead to screening policy for infection in asymptomatic man in Thailand.

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Conflicts of interest

The authors declare no conflict of interest.

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