



Prevalence of human papillomavirus and bacteria as sexually transmitted infections in symptomatic and asymptomatic women

Background: Sexually transmitted infections (STI) are common around the world, and are an important public health problem. In women, certain STIs, such as human papillomavirus (HPV), gonorrhea, chlamydia and other bacteria are often asymptomatic but can cause complications such as pelvic inflammatory disease, ectopic pregnancy, infertility, and chronic pelvic pain. This study evaluated the prevalence of HPV and other bacteria causing STIs in healthy and symptomatic women.

Methods: A total of 396 women including healthy individuals (53 symptomatic and 343 asymptomatic) were enrolled in this study. Vaginal swab samples were tested by using real-time multiplex PCR for 28 HPVs and 7 bacterial species (AnyplexII HPV28 and AnyplexII STI-7 Detection kit, Seegene, Seoul, Korea).

Results: HPV and bacteria (≥ 1 microorganisms) were detected in 25.8% and 63.1% of all subjects, respectively. In symptomatic women, the frequency of HPV and bacteria (≥ 1 microorganisms) was 35.8% (19/53) and 83.0% (44/53), respectively. HPV and bacteria (≥ 1 microorganisms) were detected in 24.2% (83/343) and 60.1% (206/343) of asymptomatic women. Detected microorganisms across subjects were as follows: *Ureaplasma parvum* (53.0%), *Mycoplasma hominis* (17.4%), *Ureaplasma urealyticum* (12.4%), *Chlamydia trachomatis* (2.8%), *Mycoplasma genitalium* (1.5%) and *Trichomonas vaginalis* (1.3%). *Neisseria gonorrhoeae* was not detected in any subjects. The frequency of bacterial detection in the symptomatic group was significantly higher than in the asymptomatic group; HPV was not differently detected in either group.

Conclusion: While HPV screening can be applied to women regardless of STI symptoms, nucleic acid amplification tests of STI bacteria may be useful for symptomatic women.

Keywords: HPV, STI, multiplex PCR, ureaplasma

Introduction

Sexually transmitted infections (STI) are common around the world, and are an important cause of morbidity and mortality [1]. Screening tests play a significant role, as STIs can be both preventable and curable; however, bacterial and viral causes of STIs are difficult to detect using conventional microbiological methods. Recently, methods have been developed and introduced to detect various microorganisms simultaneously using multiplex PCR. In particular, they are being used effectively to detect genotypes of HPV [2-4]. In addition, a kit has been developed that is capable of detecting multiple types of major STI bacteria such as chlamydia and gonorrhea, and this is currently being used to study the prevalence of such STIs in many regions [2,3,5,6]. Since HPV was discovered as a cause

of cervical cancer, screening tests, along with Papanicolaou tests, are now being performed to detect such infections [7]; however, there are no other available recommendations for the detection of STI bacteria in women.

The research subjects of this study included not only asymptomatic, healthy women but also women who had not received any previous diagnosis of a specific disease, yet had gynecological symptoms. After performing examinations on these subjects, the difference in the prevalence of HPV and STI bacteria was evaluated.

Methods

■ Materials and DNA extraction

The study was carried out in a health promotion center and women's clinic in each of

Yu Kyung Kim¹,
Dae Hyung Lee²,
Chae Hoon Lee³,
Young Seop Jung⁴,
Chang-Ho Youn⁵ &
Won Kil Lee^{*1}

¹Department of Clinical Pathology, Kyungpook National University School of Medicine, Daegu, Korea

²Department of Obstetrics and Gynecology, Yeungnam University College of Medicine, Daegu, Korea

³Department of Laboratory Medicine, Yeungnam University College of Medicine, Daegu, Korea

⁴Department of Obstetrics and Gynecology, Gimcheon Jeil Hospital, Gimcheon, Kyungpook, Korea

⁵Department of Family Medicine, Kyungpook National University School of Medicine, Daegu, Korea

*Author for correspondence:
leewk@knu.ac.kr

two university hospitals. Approval was obtained from the ethical committee of each medical center. A total of 396 women between 23 and 75 years of age were included in this study and were divided into two groups according to their symptoms and history. Healthy women without any symptoms related to gynecological disease were classified as the asymptomatic group (n=343). Fifty-three women were included in the symptomatic group as they were experiencing symptoms such as vaginal discharge, genital ulcers, or itching sensation at the time of the study. Nucleic acid extraction from collected cervical samples was conducted using MICROLAB Nimbus IVD (Hamilton, Revo, NV, USA).

■ Multiplex PCR

Multiplex PCR was conducted with the Anyplex™ II HPV28 detection kit and Anyplex™ II STI-7 detection kit (Seegene, Seoul, Korea) using a CFX96™ real-time thermocycler (Bio-Rad, Hercules, CA, USA) for individual detection of HPV and STI bacteria. The Anyplex™ II HPV28 detection kit simultaneously detects 19 high-risk HPVs (16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 69, 73, 82) and 9 low-risk HPVs (6, 11, 40, 42, 43, 44, 54, 61, 70). Seven microorganisms (i.e., *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Trichomonas vaginalis*, *Mycoplasma genitalium*, *Mycoplasma hominis*, *Ureaplasma urealyticum*, and *Ureaplasma parvum*) can be detected via the Anyplex™ II STI-7 detection kit.

Each PCR reaction was performed in 20 µL of reaction mixture containing 5 µL of extracted DNA and PCR master mix. The thermocycler conditions consisted of an initial incubation at 50°C for 4 min, denaturation at 95°C for 15 min, 50 cycles of denaturation (30 sec each at 95°C), annealing (1 min at 60°C), and extension (30 sec at 72°C).

■ Statistical analysis

Pearson χ^2 test was performed to compare

whether the differences of HPV detection rates and STI bacteria between asymptomatic and symptomatic women were significant. P values <0.05 were considered statistically significant.

Results

HPV and STI bacteria were detected in 25.8% (102/396) and 63.1% (250/396), respectively in the study subjects (TABLE 1). Among the HPV genotypes, the highest frequency was observed for type 68 (3.8%), and among STI bacteria, *Ureaplasma parvum* was detected in 53% of the study subjects (FIGURE 1A and 1B). The division of the research subjects according to their symptoms demonstrated that in HPV infection cases, 24.2% of the subjects were detected as asymptomatic and 35.8% as symptomatic; however, statistically this difference was not significant. Alternatively, STI bacteria were detected in 83.0% of symptomatic subjects, a significantly higher percentage than that of asymptomatic subjects (60.1%). Among these, the detection of *Ureaplasma parvum*, *Mycoplasma hominis* and *Mycoplasma genitalium* was significantly higher in the symptomatic group than in the asymptomatic group (TABLE 2).

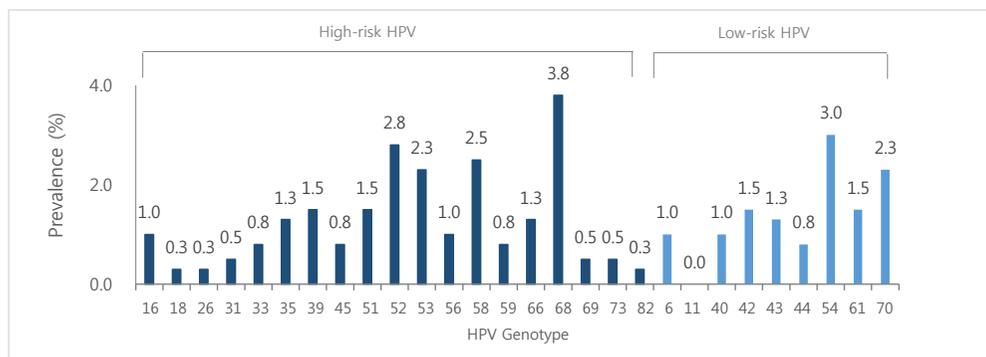
Discussion

HPV causes STI, but is also related to the development of cervical cancer. Therefore, the HPV and genotype tests are important elements of cervical cancer screening process. In this study, HPV was detected in 25.8% of the subjects, and there was no significant difference between the asymptomatic group (24.2%) and the symptomatic group (35.8%). Kim et al. [2] stated that HPV was detected in 12.1% of 799 asymptomatic women, and Chung et al. [8] also reported that the HPV prevalence rate in 518 healthy adult women was 17.6%. It is thought that the prevalence rate of the research subjects in this study was higher than that described by previous studies because the majority of the research subjects in this study were symptomatic women. Currently, the recommended practice for cervical cancer

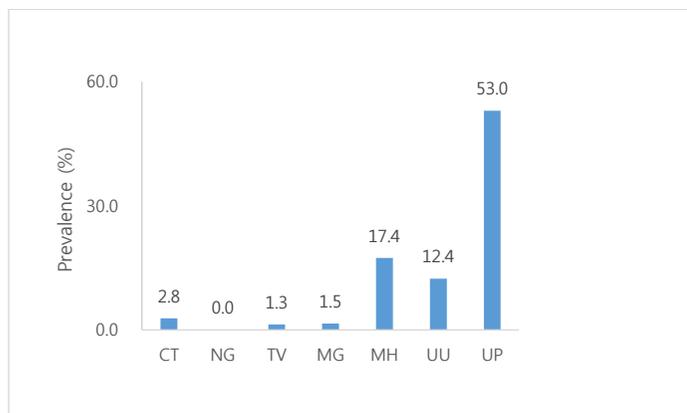
Table 1. Detection of HPV and STI bacteria in 396 Korean women.

	HPV		STI bacteria	
	Number	%	Number	%
Positive	102	25.8	250	63.1
Single	71	17.9	170	42.9
Multiple	31	7.9	80	20.2
Double	22	5.6	62	15.7
Triple	9	2.3	16	4.0
≥Quadruple	0	0.0	2	0.5

Abbreviations: HPV, human papilloma virus; STI, sexual transmitted infection



(A)



(B)

FIGURE 1. Distribution of HPV genotype (A) and STI bacteria (B). The number of microbes detected patients, including single and multiple detection was divided by total number of subjects (n=396). Abbreviations: HPV: human papilloma virus; STI: sexual transmitted infection; CT: *Chlamydia trachomatis*; NG: *Neisseria gonorrhoeae*; TV: *Trichomonas vaginalis*; MG: *Mycoplasma genitalicum*; MH: *Mycoplasma hominis*; UU: *Ureaplasma urealyticum*; UP: *Ureaplasma parvum*.

Table 2. Differences of detection rate of HPV and STI bacteria between asymptomatic and symptomatic subjects.

	Asymptomatic women (n=343)		Symptomatic women (n=53)		p*
	Number	%	Number	%	
HPV positive	83	24.2	19	35.8	NS
STI bacteria positive	206	60.1	44	83.0	0.001
HPV-STI bacteria coinfection	60	17.5	18	34.0	0.005
HPV					
High risk genotype	65	19.0	13	24.5	NS
Low risk genotype	18	5.2	6	11.3	NS
STI bacteria					
<i>Chlamydia trachomatis</i>	7	2.0	3	5.7	NS
<i>Neisseria gonorrhoeae</i>	0	0.0	0	0.0	NA
<i>Trichomonas vaginalis</i>	3	0.9	1	1.9	NS
<i>Mycoplasma genitalicum</i>	2	0.6	4	7.5	0.004
<i>Mycoplasma hominis</i>	53	15.5	15	28.3	0.021
<i>Ureaplasma urealyticum</i>	41	12.0	7	13.2	NS
<i>Ureaplasma parvum</i>	173	50.4	38	71.7	0.004

* Statistical significance was determined by the Pearson χ^2 test. Abbreviations: NS, non-specific; NA, not applicable; See Table 1.

screenings in Korea [7,8] is that asymptomatic women over the age of 20 years receive a Papanicolaou test every three years, and receive HPV examinations at a frequency based on clinical

judgment and personal preference. In United States, it is advised that an individual undergoes a Papanicolaou test every three years after they reach 21 years of age, and that they receive nucleic acid

tests every five years after 30 years of age [1]. As the prevalence rate of HPV is unrelated to the symptoms, this study suggests that nucleic acid tests regarding HPV be concurrently carried out with cervical cytology, rather than as a form of selective examination as it currently stands.

In comparison to there being no relation between the prevalence rate of HPV and whether the individual was in the symptomatic group, in the case of STI bacteria, a higher prevalence rate was discovered in symptomatic individuals than in asymptomatic individuals (symptomatic vs. asymptomatic: 83.0% vs. 60.1%). Among the 7 types of STI bacteria, the prevalence rate of *Chlamydia trachomatis*, *Neisseria gonorrhoeae* and *Trichomonas vaginalis* were extremely low or non-existent, but in the case of the United States, as chlamydia and gonorrhea are the most common forms of STD, screening inspections are recommended as a general test for both men and women [1]. In this study, *Ureaplasma parvum* was found to be the most common type of bacteria in all of the research subjects (53.0%), asymptomatic individuals (50.4%) and symptomatic individuals (71.7%). In particular, compared to the prevalence rate of the asymptomatic individuals, the prevalence rate in symptomatic individuals was significantly higher. The prevalence rate of *Ureaplasma parvum* has been previously reported in other studies with asymptomatic women as the research subjects to be 32.5% [2] and 53.1% [5] each. The infection of *Ureaplasma* during pregnancy has been reported to be related to adverse outcomes [9,10], and recently, there have been studies which state that *Ureaplasma parvum* and HPV co-infection are related to Grade 1 cervical intraepithelial neoplasia [3]. In this study, symptomatic individuals demonstrated a significantly high

prevalence rate, and it appears to be more plausible that rather than prescriptive screening tests, tests be performed after one is identified as a symptomatic individual. However, when the prevalence rate in an asymptomatic woman is 30%-50%, and in particular, where the subject is infected during pregnancy, it should be expected that these circumstances may result in adverse outcomes.

Although this study's research subjects were those without diagnosis of a particular disease, by including symptomatic women in the study, it was possible to examine the difference in prevalence rate in HPV and STI bacteria in both asymptomatic and symptomatic groups. The prevalence rate of HPV remained unchanged in both groups, thereby suggesting that cervical cancer screening tests should be performed more frequently for asymptomatic women than in current practice. In the case of STI bacteria, the prevalence rate in symptomatic individuals was higher; individuals may decide on whether to receive a screening test depending on whether they are asymptomatic or symptomatic. Pregnant women, however, should consider undergoing *Ureaplasma* tests.

Conflicts of interest

No potential conflicts of interest relevant to this article were reported.

Disclosure

No potential conflicts of interest relevant to this article were reported.

Ethics statement

The present study protocol was reviewed and approved by the institutional review board of Yeungnam University College of Medicine (Reg. No. 2014-01-368)

REFERENCES

- Yarbrough ML, Burnham CA. The ABCs of STIs: An Update on Sexually Transmitted Infections. *Clin. Chem.* 62(6), 811-823 (2016).
- Kim Y, Kim J, Lee KA. Prevalence of sexually transmitted infections among healthy Korean women: implications of multiplex PCR pathogen detection on antibiotic therapy. *J. Infect. Chemother.* 20(1),74-76 (2014).
- Drago F, Herzum A, Ciccarese G, et al. Ureaplasma parvum as a possible enhancer agent of HPV-induced cervical intraepithelial neoplasia: Preliminary results. *J. Med. Virol.* 88(12), 2023-2024 (2016).
- Marcuccilli F, Farchi F, Mirandola W, et al. Performance evaluation of Anyplex™ II HPV28 detection kit in a routine diagnostic setting: comparison with the HPV Sign® Genotyping Test. *J. Virol. Methods* 217, 8-13 (2015).
- McIver CJ, Rismanto N, Smith C, et al. Multiplex PCR testing detection of higher-than-expected rates of cervical mycoplasma, ureaplasma, and trichomonas and viral agent infections in sexually active Australian women. *J. Clin. Microbiol.* 47(5), 1358-1363 (2009).
- Kim JK. Epidemiological Trends of Sexually Transmitted Infections Among Women in Cheonan, South Korea, 2006-2012. *J. Microbiol. Biotechnol.* 23(10), 1484-1490 (2013).
- Min KJ, Lee YJ, Suh M, et al. The Korean guideline for cervical cancer screening. *J. Korean Med. Assoc.* 58, 398-407 (2015).
- Chung S, Shin S, Yoon JH, et al. Prevalence and Genotype of Human Papilloma virus Infection and Risk of Cervical Dysplasia among Asymptomatic Korean Women. *Ann. Clin. Microbiol.* 16, 87-91(2013).
- Viscardi RM. Ureaplasma species: role in neonatal morbidities and outcomes. *Arch. Dis. Child Fetal Neonatal* Ed 99, F87-F92 (2014).
- Cassell GH, Waites KB, Gibbs RS, et al. Role of Ureaplasma urealyticum in amnionitis. *Pediatr. Infect. Dis.* 5(S6), S247-S252 (1986).