Co-infections with human papillomavirus and *mycoplasma/ureaplasma* spp. in women with abnormal cervical cytology.

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Abstract

Introduction: The purpose of this study was to determine the prevalence of pathogens such as human papillomavirus (HPV) and *Mycoplasma/Ureaplasma* spp. This study investigated the association between Mycoplasma/Ureaplasma infections and HPV infections in women diagnosed with abnormal cervical cytology.

Methods: The investigation included 225 non-pregnant women diagnosed with abnormal Papanicolaou (Pap) test findings, including atypical squamous cells of undetermined significance (ASC-US), low-grade squamous intraepithelial lesion (LSIL), and high-grade squamous intraepithelial lesion (HSIL). They were analyzed between March 2010 and February 2012 according to the severity of their cervical cytology.

Results: The incidence of HPV infection was higher in the HSIL group than in the ASC-US and LSIL groups. In the HPV-positive HSIL group, Mycoplasma/Ureaplasma infections were more frequent (P<0.05), particularly Ureaplasma infections. The percentage of women infected with Mycoplasma/Ureaplasma was significantly higher in those also diagnosed with HSIL than those with LSIL or ASC-US.

Conclusion: Mycoplasma/Ureaplasma infections might be a factor of persistent infection in high-risk HPV. Since the presence of Mycoplasma/Ureaplasma was significantly associated with HPV infection, genotyping of the Mycoplasma/Ureaplasma is recommended.

Keywords: Pap test, HPV infection, *Mycoplasma/Ureaplasma*

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Introduction

Cervical cancer is a major cause of illness and death among women worldwide. In particular, human papillomavirus (HPV) infection is associated with the development of cervical cancer [1]. Cervical dysplasia and cervical cancer are caused by HPV infection. Several studies have demonstrated that fully or partially persistent HPV infection is a risk factor for cervical cancer. In addition to HPV infection, persistent infection, or an increase in viral load, other concomitant infections are also known risk factors that can cause rapid development of cervical cancer, while smoking and contraceptive medication act as environmental risk factors [2-5]. Sexually transmitted infections that have been shown to be associated with cervical cancer include chlamydia and herpes, in addition to other infectious microbes that cause concomitant infections. Chlamydia and *Mycoplasma*/*Ureaplasma* infections can cause chronic pelvic pain if the infection infiltrates the pelvis or genitourinary system. Persistent or untreated chronic infection can lead to cervical cancer in addition to pelvic pain, by causing persistent HPV infection or increased HPV levels [3].

Thus, the authors first performed HPV testing in patients with abnormal Papanicolaou (Pap) test findings in order to identify those infected with HPV. The authors investigated the HPV-positive group for the incidence of concomitant infection by *Mycoplasma*/*Ureaplasma*, which cause chronic pelvic infection, and examined the differences in the incidence of concomitant infections according to the severity of cervical cytology.

Method

The subjects consisted of patients who showed abnormal cytology in Pap tests conducted between March 2010 and December 2012 at a university hospital in Busan. HPV, *Mycoplasma,* and *Ureaplasma* infection status were verified at the same time. HPV infection status was diagnosed using the HPV DNA Hybridcapture system, while *Mycoplasma*/*Ureaplasma* were identified by culture of vaginal secretions.

Age, obstetric history, and HPV and *Mycoplasma*/*Ureaplasma* infection status were investigated retrospectively based on medical records. SPSS for Windows was used for statistical analyses. T-tests were used to compare clinical patterns and P values <0.05 were considered statistically significant. Among HPV-positive patients, concomitant infections by *Mycoplasma* and *Ureaplasma* were investigated separately.

Results

A total of 225 patients showed abnormal Pap test findings. Among these, 120 of the 192 patients (62.5%) in the atypical squamous cells of undetermined significance and low-grade squamous intraepithelial lesion (ASC-US/LSIL) group were diagnosed as being HPV-positive, while 30 of the 33 patients (90.9%) in the high-grade squamous intraepithelial lesion (HSIL) group were diagnosed as being HPV-positive. Therefore, the rate of HPV infection was higher for higher grade epithelial abnormalities (P<0.05). Notably, the rate of

concomitant *Mycoplasma* infection was also higher for higher grade lesions: 39 of 120 patients (26.7%) in the ASC-US/LSIL group and 16 of 30 patients (53.3%) in the HSIL group (P<0.05). Meanwhile, the rate of *Ureaplasma* infection was higher than the rate of *Mycoplasma* infection in both groups, but there was no significant difference between the two groups (Tables 1 and 2).

Discussion

Cervical cancer is a major cause of illness and death among women worldwide, and it is more common in developing countries [1]. HPV is a representative sexually transmitted pathogen that is strongly related to precancerous lesions of the cervix. However, HPV infection does not induce cervical carcinogenesis alone, as accompanying factors are also involved [5]. The potential accompanying factors include sexual experience at a young age (under 16 years), multiple pregnancies, multiple sexual partners, smoking, pregnancy, contraceptive medication, immunosuppressants, vitamin deficiency, low socioeconomic status, and sexually transmitted infections such as bacterial vaginosis, trichomoniasis, and chlamydia [2-5]. Thus, sexually transmitted infections play an important role in the development of dysplastic lesions of the cervix [2].

Mycoplasma species have a free lifestyle and are the smallest microorganisms to lack a cell wall. Genital *Mycoplasma* are species that are commonly found in the lower urinary tract of sexually mature men and women. Representative genital *Mycoplasma* species include *M. hominis* and *M. genitalium*, while the representative *Ureaplasma* species include *U. urealyticum*, and. A number of authors have reported that genital *Mycoplasma* cause poor pregnancy outcomes via bacterial vaginosis, pelvic inflammatory disease, infertility, premature birth, premature rupture of membranes, and chorioamnionitis [6,7]. Concomitant infection of HPV with various species of *Mycoplasma* or *Ureaplasma* has been associated with abnormal cervical cytology results in women [8]. Mohamed et al. found that women with *U. urealyticum* infection showed a significantly higher rate of abnormal cytology finding than uninfected women [9]. However, it is still unclear what effect *Mycoplasma* and *Ureaplasma* have on the natural course of cervical dysplastic lesions.

Table 1. *HPV infections among patients with abnormal Pap test results.*

	ASC-US/LSIL	HSIL	*p*-value
N	192	33	
Mean age (years)	43.2	42.9	NS
HPV infection (%)	120 (62.5)	30 (90.9)	<0.05

ASC-US: Atypical squamous cells of undetermined significance; LSIL: Low-grade squamous intraepithelial lesion; HSIL: High-grade squamous intraepithelial lesion

Table 2. *Co-infections with HPV in patients with abnormal Pap test results.*

	ASC-US/LSIL	HSIL	*p*-value
N	120	30	
Mycoplasma (%)	39 (26.7)	16 (53.3)	<0.05
Ureaplasma (%)	73 (60.8)	18 (63.3)	NS

ASC-US: Atypical squamous cells of undetermined significance; LSIL: Low-grade squamous intraepithelial lesion; HSIL: High-grade squamous intraepithelial lesion

There are several possible mechanisms by which genital *Mycoplasma* infection promotes cervical dysplasia. As an indirect mechanism, cells that have been stimulated by *Mycoplasma* show increased sensitivity to other carcinogens; in terms of direct effects, *Mycoplasma* suppresses the cell-mediated immune response, making it easier for other pathogens to persist intracellularly or to form colonies [3]. Toll-like receptors 1, 2, and 6 are expressed on the surface of antigen-presenting cells to detect the unique metabolic products of *Mycoplasma*. These receptors detect the presence of infection and induce an inflammatory response and antibacterial innate immune response, resulting in the differentiation of Th-2 polarization and the secretion of interleukin (IL)-4, -5, -10, and -13 [10]. The secreted cytokines act as antagonists to Th-1 cytokines (tumor necrosis factor [TNF]-α, IL-2, IFN-γ, IL-6, and IL-12). The imbalance in Th-1 and Th-2 cytokines impairs apoptosis and controls excessive cell differentiation by interfering with the cellular and the humoral immune responses [11,12]. Meanwhile, experimental studies have shown *Mycoplasma* infection to cause cellular mutations involving chromosomal changes, gradual loss of chromosomes, and Robertsonian translocation [13].

In the present study, the rate of *Ureaplasma* colony formation was significantly higher in the HPV-positive group than in the control group. (Additions or amendments will be made according to the study results) This result was similar to those of previous studies, in which the rate of *Ureaplasma* colony formation in women with cervical dysplasia was 32–35%, compared to 19–29.8% in non-pregnant women with normal cervical cytology and 26.3% in pregnant women with normal cervical cytology [3,14-17]. However, as mentioned by Abele-Horn et al. these results show a positivity rate of less than 70% [14]. Mohamed et al. reported *Ureaplasma* colony formation rates of 57.5% in HSIL, 36.59% in LSIL, 30.43% in ASC-US, and 21.33% in subjects with normal cytology, indicating that the rate of infection was highest in patients with HSIL. Although this is a limited result, it suggests that the interaction between HPV and *Ureaplasma* in concomitant infections plays an important role in the development of precancerous or cancerous cervical lesions) [3,9,15].

HPV infection is also a sexually transmitted infection. However, *Mycoplasma* and *Ureaplasma* infections, despite being classified as sexually transmitted, are not commonly diagnosed and treated. In addition, these bacteria can cause chronic pelvic infection resulting in chronic pelvic pain. Thus, in addition to herpes and chlamydia, which are well known, it is especially important to also test for *Mycoplasma* and *Ureaplasma* in order to determine the rate of concomitant infections in patients with abnormal cervical cytology and to be able to treat these additional factors.

Conclusion

Ureaplasma infection was more common in subjects with abnormal cervical cytology and abnormal cervical histology, while concomitant *Mycoplasma* infections were more common among subjects with abnormal findings than in those with higher grade dysplasia. This suggests that *Mycoplasma*/*Ureaplasma* infection is associated with the development of precancerous cervical lesions.

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