

Co-Infection of Human Papillomavirus with Mycoplasma Hominis/Ureaplasma Urealyticum Among Female Sex Workers in Medan, Indonesia

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Abstract

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Human papillomavirus (HPV) infection is one of the most prevalent sexually transmitted diseases among women aged < 35 years worldwide. Recent studies have suggested that the vaginal microenvironment influenced by bacterial infection poses for high-risk human papillomavirus (hrHPV) infection and cervical carcinogenesis. Female sex workers (FSWs) are a population susceptible to acquire Human Immunodeficiency Virus (HIV) and sexually transmitted infections (STIs), as well as transmitting the virus to others. The aim of this study is to evaluate the relationship between Mycoplasma/Ureaplasma infections and HPV infection among female sex workers. A total of 70 female sex workers of reproductive age were recruited from various location in Medan, Indonesia in 2018. Detection of Mycoplasma/Ureaplasma infections and HPV infection were obtained from PCR assessment. The results of this study showed that no correlation significant between Mycoplasma hominis/Ureaplasma urealyticum infection and HPV infection.

Introduction

Human papillomavirus (HPV) has been identified as an essential aetiology of warts, cervical intraepithelial neoplasia (CIN) and cervical cancer [1]. Among high-risk HPV, type 16 and 18 are the most carcinogenic for the progression of cervical disease and induce over 70% of cervical cancer, stated as the second most common cancer in women as reported in 2012 by the International Agency for Research on Cancer (IARC) [2]. The cervical cancer death currently accounted for about 57% of cases and 65% of cancer deaths worldwide [3], [4]. De Boer previously have stated that no information existed about the prevalence of HPV 18 or other HPV types in the Indonesian population and hypothesised that the high

prevalence of HPV 18 in cervical cancer in Indonesia is caused by the high prevalence of HPV 18 in the Indonesian population [5]. The estimated incidence of cervical cancer in Indonesia is 17 per 100,000 women [6].

Beside persistent infection and an increase in viral load of HPV infection, other concomitant infections are also known risk factors causing rapid development of cervical cancer, while the negative contribution of smoking and contraceptive medication act as environmental risk factors [7]. Risk factors associated with cervical cancer include first intercourse at an early age, mutually change sexual partners, and suffering from chronic sexually transmitted infections (STIs) other than HPV and HIV [8], [9], [10].

Association between HPV infection with

cervical cancer has been already known, even though several studies found *Mycoplasma hominis* and *Ureaplasma urealyticum* involvement contribute to the risk of HPV infection and the occurrence of abnormal cervical cytology [11], [12]. These bacterias were found in $30 \pm 80\%$ women's urogenital tract as a commensal organism but had the pathogenic potential [13]. Several studies revealed that *Mycoplasma hominis* and *Ureaplasma urealyticum* played a role in developing abnormal cervical cytology associated with the development of precancerous cervical lesions [7]. *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 [14].

Female sex workers (FSWs) are commonly known very vulnerable to exposure to HIV and sexually transmitted infections (STIs), as well as transmitting the virus or bacterias to others, due to behavioural risk factors as recurrent infections and repeated contact through high numbers of sexual partners [15]. This is compounded by the lack of knowledge and awareness of HPV infection, cervical cancer, and preventive efforts, as previously described in several studies [16], [17].

This study aims to evaluate the relationship between *Mycoplasma/Ureaplasma* infections and HPV infection among female sex workers in Medan, Indonesia.

Material and Methods

Study Population

This study is a descriptive study with a cross-sectional design to evaluate the relationship between *Mycoplasma hominis* and *Ureaplasma urealyticum* infection and HPV infection among female sex workers. A total of 70 female sex workers of reproductive age were recruited from various location in Medan, North Sumatera, Indonesia, between July and September 2018.

Subjects were women aged between 18 and above who were sexually active. Exclusion criteria were subjects recently diagnosed with the cervical disease within 6 months before the present study and currently pregnant. All participants were interviewed to collect information on socio-demographic background. The Ethics Committee of the University of Sumatera Utara approved the study, and informed consent forms were obtained from all subjects.

Detection of HPV and *Mycoplasma/Ureaplasma*

DNA samples were isolated from cervical swabs using Prestoⁿ Buccal Swab gDNA extraction kit. DNA was then amplified using the established PCR method. PCR kit components used for HPV gene amplification were DNA template of 6 μ l, *GoTaq[®] Green Master Mix (Promega)* of 12.5 μ l, a primer of forwarding HPV. *Mycoplasma hominis* and *Ureaplasma urealyticum* used 2 primers: RNAH1 and RNAH2 that amplify the 16sRNA *Mycoplasma hominis* gene at 334 bp; UMS125 and UMA226 amplified *Ureaplasma urealyticum* serovar 3 which shows multiple bands at 403 bp for biovar 1 and 448 bp for biovar 2. The amplification mixture was carried out in 12.5 μ l master mix PCR consisted of Taq polymerase enzyme, MgSO₄, and dNTP(*Go Taq[®] PCR Core System, Promega*).

Results

The data summarised in Table 1 showed the distribution of age according to the infection of *Mycoplasma hominis/Ureaplasma urealyticum* and HPV. This study showed that HPV 16 infection was higher in women aged 26-30 (12.5%), while that HPV 18 infection was found higher in women aged 18-25 (26.9%). The occurrence of *Mycoplasma* infections was more common in the age group 18-25 (31.4%), while *Ureaplasma* infections were more prevalent in the age group 31-45 (60.9%).

Table 1: Distribution of age by infection of *Mycoplasma hominis/ Ureaplasma urealyticum* and HPV

Infection	Total N = 70	Age in years			
		18-25 N = 26 (37.1%)	26-30 N = 16 (22.9%)	31-45 N = 23 (32.9%)	> 45 N = 5 (7.1%)
HPV16 (+)	3(4.3)	1(3.8)	2(12.5)	0(0)	0(0)
HPV16 (-)	67(95.7)	25(96.2)	14(87.5)	23(100)	5(100)
HPV18 (+)	13(18.6)	7(26.9)	3(18.8)	3(13.0)	0(0)
HPV18 (-)	57(81.4)	19(73.1)	13(81.3)	20(87.0)	5(100)
<i>Mycoplasma</i> (+)	22(31.4)	11(42.3)	5(31.3)	6(26.1)	0(0)
<i>Mycoplasma</i> (-)	48(68.6)	15(57.7)	11(68.8)	17(73.9)	5(100)
<i>Ureaplasma</i> (+)	32(45.7)	9(34.6)	6(37.5)	14(60.9)	3(60.0)
<i>Ureaplasma</i> (-)	38(54.3)	17(65.4)	10(62.5)	9(39.1)	2(40.0)

The previous study found that infections of *C. trachomatis*, *M. genitalicum*, and *U. parvum* were higher in the younger age group by comparisons based on age (< 50 years vs \geq 50 years) [10]. Similar to the previous study, the respondents of this study mostly younger than 50 years, and bacterial infections more often to occur in the age group 18-25 years and 31-45 years.

Another study found that HPV infection peak in women aged 40-50 years [18]. Different from the results of the former study, HPV 16 infections were higher in women aged 26-30 years, while HPV 18 found high in women aged 18-25 years. Nevertheless,

another study showed no correlation between HPV infection and the age of the respondent, and also found that age was not related to abnormal cytology or bacterial infection [19].

Table 2: Relationship between Mycoplasma hominis and Ureaplasma urealyticum infection and HPV infection

Bacterial infection	HPV 16 infection			OR	P-value	CI 95%	HPV 18 infection				
	Positive	Negative					Positive	Negative	OR	P-value	CI 95%
Mh Positive	0 (0,0%)	22 (32,8%)		0.547 ^a	-	-	3 (23,1%)	19 (33,3%)	0.6	0.742 ^a	0.148-2.440
Mh Negative	3 (100,0%)	45 (67,2%)					10 (76,9%)	38 (66,7%)			
Uu Positive	2 (66,7%)	30 (44,8%)	2.426	0.589 ^a	0.213-28.535	6 (46,2%)	26 (45,6%)	1.0	1.000 ^a	0.305-3.422	
Uu Negative	1 (33,3%)	37 (55,2%)					7 (53,8%)				31 (54,4%)

The results of this study as shown in Table 2, found that no correlation between Mycoplasma hominis infection with HPV 16 infection ($p = 0.547$) and neither with HPV 18 infection ($p = 0.742$). There were no correlation as well between Ureaplasma urealyticum infection with HPV 16 infection ($p = 0.580$), and also no correlation with HPV 18 infection ($p = 0.639$).

Discussion

The results were different from previous studies that found a significant association between HPV infection and the presence of Mycoplasma/Ureaplasma infection [11], [18]. Another study showed the correlation not only between bacterial infection and high-risk HPV infection but also between abnormal cytology with bacterial infection [10]. Another previous study from Korea from asymptomatic women of reproductive age with negative cytologic findings found that most HPV infections were not correlated with any specific STIs [20]. Another study from Estonia found the high risk-HPV was associated with STIs especially Chlamydia infection, and also with U. urealyticum infection in women over 41 years old. The same study found that U. urealyticum infection was not associated with HPV status [21]. A study from Nigeria found significant associations between persistent high-risk HPV infections and persistent M. Hominis in the vaginal microbiota, and the study suggested that M. Hominis may play a role in high-risk HPV induced cervical carcinogenesis [22].

Several studies showed that a high density of Ureaplasma might be associated with STIs and the differentiation of colonisation and infection contributed to co-infection, as reported by Kim et al., that showed only Ureaplasma colonization with greater than 104 CCU/mL was significantly associated with HPV infection [19]. Another study which in line showed patients with cervical lesions had higher U. parvum load colony-forming unit [23]. Unfortunately, in this study, we did not assess the bacterial load of

Ureaplasma and Mycoplasma. The correlation between colonies number and infection may be explained by more pathogen colony could develop more cervical lesion as well due to chronic infections by activating the virulence factor. Few studies have been conducted to evaluate the association between ureaplasma load with cervical disorders especially with precursor cervical lesions of cervical cancer [5].

In conclusion, this study revealed that there is no significant correlation between the presence of Mycoplasma hominis/Ureaplasma urealyticum infection and HPV infection. Although among female sex workers, sexually transmitted infections like Mycoplasma spp, and Ureaplasma spp were frequently detected and as well as the HPV infection. However, this non-significant result might be due to the limitation of this study. We only recruited a small number of samples and therefore could not provide confirmed statistical significance for all of the tests performed.

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