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著者	Hoang Huyen Thi Thanh, Ishizaki Azumi, Nguyen Cuong Hung, Tran Vuong Thi, Matsushita Kaori, Saikawa Kunikazu, Hosaka Norimitsu, Pham Hung Viet, Bi Xiuqiong, Ta Van Thanh, Pham Thuc Van, Ichimura Hiroshi
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## **Infection with High-risk HPV Types among Female Sex Workers in Northern Vietnam**

Huyen Thi Thanh Hoang<sup>1,2,3</sup>, Azumi Ishizaki<sup>1</sup>, Cuong Hung Nguyen<sup>1,2</sup>, Vuong Thi Tran<sup>1,2</sup>,  
Kaori Matsushita<sup>1</sup>, Kunikazu Saikawa<sup>4</sup>, Norimitsu Hosaka<sup>1,5</sup>, Hung Viet Pham<sup>1</sup>, Xiuqiong  
Bi<sup>1</sup>, Van Thanh Ta<sup>3</sup>, Thuc Van Pham<sup>1</sup>, and Hiroshi Ichimura<sup>1\*</sup>

<sup>1</sup>Department of Viral infection and International Health, Graduate school of Medical Science,  
Kanazawa University, Kanazawa, Japan; <sup>2</sup>Hai Phong Medical University, Hai Phong,  
Vietnam; <sup>3</sup>Hanoi Medical University, Hanoi, Vietnam; <sup>4</sup>Department of Human Pathology,  
Graduate School of Medical Science, Kanazawa University, Kanazawa, Japan; <sup>5</sup>Eiken  
Chemical Co., Ohtawara, Tochigi, Japan.

**\*Corresponding author:** Hiroshi Ichimura, M.D., Ph.D.

Department of Viral infection and International Health, Graduate school of Medical  
Science, Kanazawa University.

13-1 Takaramachi, Kanazawa 920-8640, Japan.

Tel.: +81 76 265 2228; fax: +81 76 234 4237.

E-mail address: [ichimura@med.kanazawa-u.ac.jp](mailto:ichimura@med.kanazawa-u.ac.jp)

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## ABSTRACT

Vaccines against two high-risk human papillomavirus (HPV) types, HPV-16 and HPV-18, are in use currently, with high efficacy in preventing infections with these HPV types and consequent cervical cancers. However, circulating HPV types can vary with geography and ethnicity. The aim of this study was to investigate the prevalence of HPV types and the association between HPV types and abnormal cervical cytology among female sex workers in Northern Vietnam. Cervical swabs and plasma samples were collected from 281 female sex workers at two health centers in Hanoi and Hai Phong in 2009. The HPV L1 gene was amplified by PCR using original and modified GP5<sup>+</sup>/6<sup>+</sup> primers. Amplified PCR products were genotyped by the microarray system geneSQUARE (KURABO) and/or clonal sequencing. Of the 281 women, 139 (49.5%) were positive for HPV DNA. Among the HPV-positive samples, 339 strains and 29 different types were identified. Multiple-type and high risk-type HPV infections were found in 85 (61.2%) and 124 (89.2%) women, respectively. The most common genotype was HPV-52, followed by HPV-16, HPV-18, and HPV-58. Abnormal cervical cytology was detected in 3.2% (9/281) of the women, and all of these samples were positive for HPV-DNA. Age  $\leq$  25 years and infection with human immunodeficiency virus were associated positively with HPV infection among the women while ever smoking was associated negatively. These results show that HPV-52 is most prevalent among female sex workers in Northern Vietnam, most of whom had normal cervical cytology. This information might be important for designing vaccination strategies in Vietnam.

## INTRODUCTION

Genital human papillomavirus (HPV) infection is the most common infection transmitted sexually among women and the main cause of cervical cancer worldwide, especially in developing countries, where 85% of cervical cancer cases occur [Ferlay et al., 2010]. There were an estimated 529,000 new cases and 275,000 cervical cancer-related deaths globally in 2008, including 312,000 (59%) new cases in Asia, and HPV infections are prevalent particularly in South and Southeast Asia [Ferlay, 2010; WHO, 2010]. Cervical cancer screening using cytological testing and HPV vaccination are of paramount importance for preventing cervical cancer in young women.

HPV belongs to the family Papillomaviridae. More than 100 distinct HPV genotypes have been characterized molecularly, and about 40 HPV types have been identified in the mucosal epithelia of the human genital tract [Munoz et al., 2006]. Cervical cancer is caused by HPV types that belong to a few “high-risk” species of the mucosotropic alpha genus, such as alpha-5, -6, -7, -9, and -11 [Bouvard et al., 2009; Schiffman 2010]. Eight HPV types (HPV-16, -18, -31, -33, -35, -45, -52, and -58) are observed most frequently and are responsible for about 90% of all cases of cervical cancer worldwide [Munoz et al., 2006]. In particular, HPV-16 and HPV-18 are observed in 70% of cervical cancer cases worldwide [Munoz et al., 2003; Clifford et al., 2006].

Current prophylactic HPV vaccines targeting HPV-16 and HPV-18 hold great promise for reducing the global burden of cervical cancer [Harper et al., 2006; Wheeler, 2007]. However, circulating HPV types can vary by geography and ethnicity, and the current vaccine formulary for these two high-risk types is less effective against some other oncogenic HPV types, although a recent study has shown cross-protective efficacy of the HPV-16/18

vaccine against oncogenic HPV types such as HPV-31, HPV-33, HPV-45, and HPV-51 [Wheeler, 2012]. In Europe and America, HPV-16 and HPV-18 are the most common HPV types [Clifford et al., 2005] whereas in Asia, in addition to HPV-16, HPV-52 and HPV-58 are most common [Bao et al., 2008]. Particularly in Japan, the Philippines, Taiwan, and the Zhejiang province of southeast China, HPV-52 is reported to be the HPV type identified most frequently [Lin et al., 2006; de Sanjosé et al., 2007; Miyashita et al., 2009; Ye et al., 2010]. Thus, an understanding of the geographical distribution of HPV types is necessary to estimate vaccine efficacy accurately and prevent HPV infection and the subsequent development of cervical cancer.

In Vietnam, more than 6,000 new cases of cervical cancer (incidence rate: 11.7 per 100,000 women per year) and 3,000 cervical cancer-related deaths are estimated to occur each year. Cervical cancer ranks as the second most common cancer in women ages 15–45 years [Domingo et al., 2008]. Screening for cervical cancer with the Pap smear test and HPV DNA detection are not available widely in Vietnam [WHO, 2002]. Previous studies identified HPV-16 and HPV-58 as the most common high-risk HPV types in a general population of Vietnamese women [Pham et al., 2003; Domingo et al., 2008] while another study reported that HPV-52 was the most common type among female sex workers in Southern Vietnam [Hernandez et al., 2008]. However, population-based information on the distribution of HPV types among Vietnamese women is limited still [Bao et al., 2008]. In the current study, the prevalence of HPV infection, the distribution of HPV types, and risk factors for HPV infection among female sex workers in Northern Vietnam were determined. The association between HPV types and abnormal cervical cytology was also investigated.

## **SUBJECTS AND METHODS**

### **Subjects and Sample Collection**

A cross-sectional survey of HPV infection and genotype distribution among female sex workers in Northern Vietnam was conducted from June to November 2009. The 281 participants (mean age  $\pm$  SD: 27.6  $\pm$  8.0 years) had been commercial sex workers previously and were concentrated in two rehabilitation centers in Hanoi and Hai Phong, the largest cities in Northern Vietnam. They were recruited after giving written informed consent. A gynecological examination was performed, and two cervical-swab samples were collected using a cervical brush (Honest Uterine Cervical Brushes; Honest Medical, Tokyo, Japan). The cervical swabs were smeared onto a slide, fixed with alcohol solution (Rapid Fix; Muto, Tokyo, Japan), and stained according to standard procedures for the Pap smear test. The remainder of each sample was suspended in 1 ml of lysis buffer (TBE buffer, 50 mM Tris-HCl, 5 mM EDTA, 2% SDS) and stored at  $-80^{\circ}\text{C}$  until use. Sociodemographic information was collected using questionnaires. Blood samples were collected and plasma samples stored at  $-80^{\circ}\text{C}$  until use. The study protocol was reviewed and approved by the board of the Ministry of Health of Vietnam and by the ethics committee of Kanazawa University, Japan.

### **DNA Extraction**

Genomic DNA was extracted from cervical cells in lysis buffer using a DNA extraction kit (SMI Test; Genome Science Laboratories, Fukushima, Japan) according to the manufacturer's instructions. The quality of the extracted DNA was evaluated by amplifying the glyceraldehyde-3-phosphate dehydrogenase gene (primers: 5'-ACCACAGTCCATGCCATCAC-3' and 5'-TCCACCACCCTGTTGCTGTA-3') (Fujimori et

al., 2002). All extracted DNA samples were confirmed as adequate for HPV, *Chlamydia (C.) trachomatis*, and *Neisseria (N.) gonorrhoeae* testing.

### **HPV Detection**

HPV DNA was detected by PCR using three pairs of modified GP5<sup>+</sup>/6<sup>+</sup> primers: GP5<sup>+</sup>M1-2 (5'-TTTRTTACTGTTGTWGATACTAC-3') and GP5<sup>+</sup>M2-2 (5'-TGTWACTGTTGTWGATAACCAC-3'); GP5<sup>+</sup>M3-2 (5'-GTWACTGTTGTRGACACCAC-3') and GP6<sup>+</sup>M1-2 (5'-AATTGAAAWATAAACTGTAAWTCATATTC-3'); and GP6<sup>+</sup>M2-2 (5'-GAAACATAAAAYTGTAATCAWATTC-3') and GP6<sup>+</sup>M3 (5'-GAAAATYTGCAAATCAWACTC-3') [Yamada et al., 2008; Miyashita et al., 2009]. These modified GP5<sup>+</sup>/6<sup>+</sup> primers were designed to minimize mismatches between primer sequences and complement target HPV L1 genes and to amplify a 140-bp fragment of the HPV L1 gene. Amplification was performed as follows: one cycle at 95°C for 10 min, followed by 45 cycles at 95°C for 30 s, 45°C for 30 s, and 74°C for 30 s, with a final extension at 74°C for 10 min. The presence of HPV DNA was confirmed by ethidium bromide staining of the PCR products following agarose gel electrophoresis. PCR was repeated using the original GP5<sup>+</sup>/GP6<sup>+</sup> primers for the HPV DNA–negative samples [de Roda Husman et al., 1995].

### **HPV Genotyping**

HPV genotyping was performed with a DNA microarray system, KURABO GeneSquare microarray (KURABO, Okayama, Japan), which uses multiplex PCR targeting different genes from type to type [Ermel et al., 2010]. The sensitivity and specificity of the

GeneSquare is equal reportedly to that of the Roche Linear Array HPV Genotyping Assay.

The GeneSquare microarray contains 23 type-specific probes: HPV-6, -11, -16, -18, -30, -31, -33, -34, -35, -39, -40, -42, -45, -51, -52, -53, -54, -56, -58, -59, -61, -66, and -68.

The original and/or modified GP5<sup>+</sup>/6<sup>+</sup> PCR products of the samples that were not genotyped by the GeneSquare microarray were cloned using the TOPO TA Cloning Kit (Invitrogen, Carlsbad, CA, USA) and sequenced according to the manufacturer's instructions. The similarity between the L1 sequences obtained from the PCR products and those of various HPV genotypes registered in the GenBank database was determined by BLAST analysis (<http://www.ncbi.nlm.nih.gov/BLAST/>).

HPV types that belong to the mucosotropic alpha genus were classified as high-risk types (HPV-16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -59, -68) or possibly high-risk types (HPV-26, -53, -66, -67, -69, -70, -73, -82, -85, -97) according to the classification of the International Agency for Research on Cancer [Bouvard et al., 2009], or as low-risk types (HPV-6, -11, -40, -42, -43, -44, -54, -55, -61, -62, -72, -81, -83, -84, -89) [Munoz et al., 2003] or unknown-risk types (HPV-2, -3, -7, -10, -13, -27, -28, -29, -30, -32, -34, -55, -57, -62 -71, -74, -77, -78, -85, -86, -87, -90, -91, 94, 102, 106) [Schiffman et al., 2010].

### **Detection of Infections Transmitted Sexually or Blood Borne**

Anti-human immunodeficiency virus (HIV; a member of the lentivirus genus in the family Retroviridae) antibody, anti-hepatitis C virus (HCV; a member of the hepacivirus genus in the family Flaviviridae) antibody, and hepatitis B virus (HBV; a member of the orthohepadnavirus genus in the family of Hepadnaviridae) surface antigen were tested using serological test kits



(Abbott, Tokyo, Japan). *C. trachomatis* and *N. gonorrhoeae* were detected using the loop-mediated isothermal amplification method [Hong et al., 2004; Poon et al., 2005].

### **Classification of Cervical Cytology**

The Bethesda Reporting System 2001 was used to classify cervical cytology [Solomon et al., 2002] as normal (negative for intraepithelial lesion or malignancy), atypical glandular cells/atypical squamous cells of undetermined significance, low-grade squamous intraepithelial lesion, high-grade squamous intraepithelial lesion, or adenocarcinoma in situ.

### **Statistical Analysis**

Statistical analysis was performed using SPSS Version 19.0 for Windows. The chi-square test and/or Fisher's exact test were used for comparisons between HPV DNA-positive and –negative groups, and univariate analysis was performed to assess the association between HPV DNA-positive results and risk factors or other infections. A multivariate analysis was performed by using a stepwise binary logistic regression model to confirm the association. *P* values of  $\leq 0.05$  were considered to indicate statistical significance.

## **RESULTS**

### **Profile of Cervical HPV Infection**

Of the 281 women, 139 (49.5%) were positive for HPV DNA. Of these, 54 (38.8%) were infected with a single HPV type, and 85 (61.2%) were infected with multiple types. Multiple-type HPV infection was more common in women  $\leq 25$  years than in those  $> 25$  years old (OR: 2.1; 95% CI: 1.3–2.6), and also more common in women infected with HIV than in women without HIV infection (OR: 2.2; 95% CI: 1.1–4.4). From the 139 HPV DNA–positive samples, 339 HPV strains and 29 different genotypes were isolated (Figure 1). Of the 339 strains, 228 (67.3%) were high-risk, 27 (8.0%) possible high-risk, 54 (15.9%) low-risk, and 30 (8.8%) unknown-risk HPV types. Infection with high-risk HPV types was found in 89.2% (124/139) of the women infected with HPV. Among the high-risk HPV types, HPV-52 was the most common type (28.1%; 39/139), followed by HPV-16 (18.7%; 26/139), HPV-18 (16.5%; 23/139), HPV-51 (16.5%; 23/139), and HPV-58 (16.5%; 23/139).

### **Prevalence of Infections Transmitted Sexually or Blood Borne**

The 281 women were tested for infections transmitted sexually or blood borne, such as infections with HIV, HBV, HCV, *N. gonorrhoeae*, and *C. trachomatis*. Of the 281 women, 177 (63.0%) had at least one infection. The prevalence of these infections was 12.8% (36/281) for HIV-1, 6.8% (19/281) for HBV, 18.5% (52/281) for HCV, 1.4% (4/281) for *N. gonorrhoeae*, and 6.8% (19/281) for *C. trachomatis*.

### **Risk Factors for Cervical HPV Infection**

To determine the risk factors associated with cervical HPV infection, a univariate analysis was performed. Age  $\leq$  25 years (OR: 2.2; 95% CI: 1.4–3.6), being single (OR: 1.9; 95% CI: 1.2–3.1), smoking (OR: 0.6; 95% CI: 0.3–0.9), and HIV infection (OR: 4.2; 95% CI: 1.2–3.1) were associated significantly with HPV infection (Table I). When all of the variables were adjusted for multivariate analysis, age  $\leq$  25 years (OR: 2.3; 95% CI: 1.4–3.9), smoking (OR: 0.5; 95% CI: 0.3–0.8), and HIV infection (OR: 7.9; 95% CI: 3.1–20.2) were confirmed as independent factors predicting high-risk HPV infection.

### **Association between High-risk HPV Types and Abnormal Cervical Cytology**

Of the 281 women, 272 (96.8%) had normal cervical cytology, four (1.4%) had atypical glandular cells/atypical squamous cells of undetermined significance, and five (1.8%) had abnormal cervical cytology (four with a low-grade squamous intraepithelial lesion and one with a high-grade squamous intraepithelial lesion) with the Pap smear test. HPV DNA was detected in the four women with atypical glandular cells/atypical squamous cells of undetermined significance, as well as in the five women with abnormal cervical cytology. All of them were infected with high-risk HPV types (Table II).

### **Sequence Data**

The sequences described in this report have been deposited in GenBank/EMBL/DDBJ under accession numbers AB706253–AB706269.

## DISCUSSION

In the current study, the prevalence of cervical HPV infection among female sex workers in Northern Vietnam was 49.5%. This value is lower than the prevalence reported for a similar population in Southern Vietnam (85%) in 2008 [Hernandez et al., 2008]. The difference may reflect the previous finding that HPV prevalence among the general population of women in Southern Vietnam is 5-fold higher than in Northern Vietnam [Pham et al., 2003].

Nevertheless, the HPV prevalence in this study is similar to that among female sex workers in other Asian countries, such as the Philippines (57.2%) [Miyashita et al., 2009], Japan (52.6%) [Matsushita et al., 2011], and Korea (47%) [Choi et al., 2003]. A similar HPV prevalence among female sex workers was reported in Kenya (55.6%) [Luchters et al., 2010], Tunisia (44.1%) [Znazen et al., 2010], Peru (50.6%) [Montano et al., 2011], and Mexico (48.9%) [Juárez-Figueroa et al., 2001]. A lower HPV prevalence among female sex workers was reported in Spain (39%) [del Amo et al., 2005] and Australia (32%) [Tideman et al., 2003].

HPV-52 was found to be the most prevalent HPV type among female sex workers in Northern Vietnam, most of whom had normal cervical cytology. This result is consistent with those of a previous study of female sex workers in Southern Vietnam [Hernandez et al., 2008], as well as with results from other Asian regions, such as South Taiwan [Lin et al., 2006], the Philippines [Miyashita et al., 2009], and Japan [Matsushita et al., 2011]. These findings suggest that HPV-52 is common in Asian countries in general, although HPV-16 has been reported to be the most prevalent type in Asia except for Japan and Taiwan [de Sanjose et al., 2007; Bruni 2010].

It was reported previously that HPV-16 is the most common HPV type among the general population of Vietnamese women; those results were obtained using the original

GP5<sup>+</sup>/GP6<sup>+</sup> primers and an enzyme immunoassay for genotyping [Pham et al., 2003]. The difference between these previous results and those reported here might result from the primer set used for HPV PCR. Although the original GP5<sup>+</sup>/6<sup>+</sup> primer set has been used in many epidemiological studies, it does not amplify HPV-52 as effectively as HPV-16 and HPV-18 because of sequence mismatches between the target gene and the primers [Yamada et al., 2008, Miyashita et al., 2009]. Thus, studies using only the GP5<sup>+</sup>/6<sup>+</sup> primer set could have underestimated HPV-52 prevalence. In this study, modified GP5<sup>+</sup>/6<sup>+</sup> primer sets were used together with the original set to broaden the spectrum of detectable HPV types [Miyashita et al., 2009].

In the current study, the risk of HPV infection was significantly higher in women  $\leq 25$  years than in women  $> 25$  years of age (OR = 2.1, 95% CI: 1.4–3.9). This finding is consistent with previous reports that the prevalence of HPV is age dependent, with a peak in young women after the onset of sexual activity [Molano et al., 2003; Miyashita et al., 2009]; one explanation might be that in young women, there is a higher probability of exposure to HPV and less acquired immunity to HPV from past exposure. Thus, younger women would benefit more from HPV vaccination programs.

Smoking increases the risk of squamous-cell carcinoma of the cervix. However, previous studies have found a negative [Ho et al., 1998], positive [Minkoff et al., 2004; Pista et al., 2012], or null [Vaccarella et al., 2008] association between smoking and HPV infection over time. In this study, ever smoking was associated with a lower risk of HPV infection, although the protective mechanism of smoking against HPV infection and whether it is a biologic or a confounding effect are unknown.

Among the female sex workers in Northern Vietnam, HPV infection was the most prevalent (49.5%), followed by HCV (18.5%), HIV (12.8%), HBV (6.8%), *C. trachomatis* (6.8%), and *N. gonorrhoeae* (1.4%) infections. Of these infections, only HIV infection had a significantly higher association with HPV infection in the women in the current study (OR: 7.9; 95% CI: 3.1–20.2), which is consistent with previous reports that HIV-related immunosuppression increases the risk of genital HPV infection and affects HPV replication [Ho et al., 1994]. Humoral immune responses are altered within a few months after HIV infection [Marais et al., 2009], which may reduce the ability of women infected with HIV to produce HPV-specific secretory IgA antibodies, resulting in an increase in the HPV infection rate. It was also reported that HPV infections are more likely to persist in women infected with HIV compared to women not infected with HIV [Clifford et al., 2006; Luchters et al., 2010].

In this study, the Pap smear test revealed that only nine women (3.2%) had “abnormal” cervical cytology (four with atypical glandular cells/atypical squamous cells of undetermined significance, four with a low-grade squamous intraepithelial lesion, and one with a high-grade squamous intraepithelial lesion). The frequency of abnormal cervical cytology in this study is much lower than that among female sex workers in the Philippines (15.2%)[Miyashita et al., 2009] and Japan (12.8%)[Matsushita et al., 2011]. The difference might be due to whether those study subjects were active female sex workers (in the studies of the Philippines and Japan) or not (in this study). However, considering that the HPV prevalence in this study is similar to that among female sex workers in those previous studies [Miyashita et al., 2009; Matsushita et al., 2011], the possibility that collection and fixation of cervical swab samples were done under limited conditions, which affected the Pap smear

analysis, could not be excluded completely in this study. All nine of the women with abnormal cervical cytology were infected with one or more high-risk HPV types. Thus, the correlation between infection with high-risk HPV types and abnormal cervical cytology was confirmed. Although population-based Pap smear screening has been introduced in Vietnam, only 4.9% of the general population of women ages 18–69 years have undergone the screening [WHO, 2010], and cervical cancer has become progressively a leading cause of cancer-related death among women in Vietnam [Domingo et al., 2008]. Considering these data, an active program is needed to control cervical cancer effectively using not only the Pap smear test but also the cervical HPV DNA test in Vietnam.

In conclusion, HPV-52 was the most prevalent high-risk HPV type among female sex workers in Northern Vietnam, most of whom had normal cervical cytology. Age  $\leq 25$  years and HIV infection were associated positively with HPV infection among the women and ever smoking was associated negatively. These findings suggest that the current HPV vaccines targeting HPV-16 and HPV-18 may not be sufficient to prevent infection with high-risk HPV types in this region. Thus, second-generation HPV prophylactic vaccines that include HPV-52 might be necessary to prevent HPV infection in Northern Vietnam. However, the clinical relevance of the use of new vaccines that include other high-risk types such as HPV52 was not demonstrated in this work. Even though HPV52 was the most prevalent type, it was not found to be related to a high-grade squamous intraepithelial lesion, but was HPV16. It is, therefore, important to investigate the prevalence of HPV-52 among patients with cervical cancer in this geographic area, which is ongoing currently.

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## **FIGURE LEGEND**

Figure 1: Prevalence of HPV genotypes among female sex workers in Northern Vietnam.



TABLE I. Risk factors associated with HPV infection.

		FSW <sup>a</sup>			Univariate			Multivariate		
		cases	HPV DNA (+) <i>n</i>	%	OR <sup>b</sup>	95% CI	<i>P</i>	OR <sup>b</sup>	95% CI	<i>P</i>
Age (years)	≤25	138	82	59.4	2.2	1.4–3.6	0.001	2.3	1.4–3.9	0.001
	>25	143	57	39.9	1					
Age at first sexual encounter (years)	<18	117	61	52.1	1.2	0.7–1.9	0.467			
	≥18	160	76	47.5	1					
Marital status	Single	139	80	57.6	1.9	1.2–3.1	0.009			
	Married/ cohabiting	142	59	41.5	1					
Education level	≥ Secondary	193	98	50.8	1.2	0.7–2.0				
	< Secondary	88	41	46.6	1					
Pregnancy history	Yes	203	95	46.8	0.7	0.4–1.2	0.183			
	No	78	44	56.4	1					
Contraception use	Yes	227	114	50.2	1.1	0.6–2.1	0.761			
	No	53	25	47.2	1					
Condom use	Yes	133	63	47.4	0.8	0.5–1.3	0.476			
	No	147	76	51.7	1					
Smoking	Ever	101	41	40.6	0.6	0.3–0.9	0.025	0.5	0.3–0.8	0.006
	Never	178	98	55.1	1					
HIV	Positive	36	28	77.8	4.2	1.9–9.6	<0.001	7.9	3.1–20.2	<0.001
	Negative	245	111	45.3	1					
HBV	Positive	19	11	57.9	1.4	0.6–3.7	0.484			
	Negative	262	128	48.9	1					
HCV	Positive	52	25	48.1	0.9	0.5–1.7	0.878			
	Negative	228	114	50.0	1					
<i>N. gonorrhoeae</i>	Positive	4	3	75	3.1	0.3–30.3	0.367			
	Negative	277	136	49.1	1					
<i>C. trachomatis</i>	Positive	19	13	68.4	2.3	0.9–6.3	0.1			
	Negative	262	126	48.1	1					

<sup>a</sup>FSW: female sex workers. <sup>b</sup>Odds ratio adjusted for all variables.

HBV: hepatitis B virus; HCV: hepatitis C virus; HIV: human immunodeficiency virus; HPV: human papillomavirus

TABLE II. Relationship between HPV genotypes and cervical cytology in female sex workers in Northern Vietnam.

Cervical cytology ( <i>n</i> )	Sample ID	Age	Smoking	Infection	HPV Types	
					Low risk	High risk
Atypical squamous cells of undetermined significance (2)	HPV-2-118	28	No			39, 68
	HPV-2-201	21	Yes	HIV, HCV		59
Atypical glandular cells (2)	HPC-028-09	29	No	HIV		16, 52
	HPV-2-176	23	No	<i>N. gonorrhoeae</i> <i>C. trachomatis</i>		16, 33, 51, 52, 53, 58
Low-grade squamous intraepithelial lesion (4)	HPV-2-119	17	No	HIV <i>C. trachomatis</i>	6, 42, 54	35, 51, 68
	HPV-2-124	30	Yes	HIV, HCV		66
	HPV-2-127	27	Yes			51,52
	HPV-2-130	22	No	<i>C. trachomatis</i>	40	51,66
High-grade squamous intraepithelial lesion (1)	HPC-048-09	48	Yes			16

HIV: human immunodeficiency virus; HPV: human papillomavirus; HCV: hepatitis C virus;

*N. gonorrhoeae*: *Neisseria gonorrhoeae*; *C. trachomatis*: *Chlamydia (C.) trachomatis*.

