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Prevalence of Human Papillomavirus and Herpes Simplex Virus Type 2 Infection in Korean Commercial Sex Workers

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In order to investigate the prevalence of sexually transmitted viruses such as human papillomavirus (HPV) and herpes simplex virus (HSV) in Korean commercial sex workers (CSWs), we selected 188 CSWs (age range 20–44 years, median age 24 years) who regularly visited one public health center in Seoul, Korea. HPV genotypes were analyzed by using a HPV DNA Chip, and an enzyme-linked immunosorbent assay (ELISA) was used to detect type-specific IgG against HSV2 antibody identifying seropositivity for HSV2 infection. Polymerase chain reaction (PCR) was performed with specific primers to detect HPV and HSV1/2 in cervical swabs from the CSWs. The prevalence of HPV infection was 83.5% in 188 cervical swab specimens and the main high-risk HPV genotypes were HPV16, 18, 56, and 58. The principal low-risk HPV genotypes were HPV6 and 11. The prevalence of HSV1/2 DNA was 13.8% and HSV2 seroprevalence was 86.2%. These results suggest that high frequencies of HPV and HSV2 infection might contribute to the rapid spread of STD viruses in CSWs in Korea. Additionally, an understanding of why high-risk HPV genotypes are so prevalent could provide guidelines for prophylactic vaccine development in Korea.

Keywords: Human papillomavirus, cervical swab, commercial sex workers, herpes simplex virus

Human papillomavirus (HPV) infection is a very strong and independent predictor of the presence of squamous intraepithelial lesions (SIL) and invasive cancer of the uterine cervix [22]. The approximately 45 HPV types that infect genital mucosa are classified as low-risk HPV (LR HPV) or high-risk (HR HPV) based on their association with premalignant (LR HPV) or malignant (HR HPV) lesions [1]. HPV infection is the main cause of invasive cervical cancer, although cofactors such as smoking, multiparity, long duration of oral contraceptive use, and herpes simplex virus type 2 (HSV2) infection may act in conjunction with HPV to facilitate cancer development [6]. Herpes simplex virus (HSV) infection was earlier considered a possible causal agent of cervical cancer [15]. An epidemiological study found that women infected with both HSV2 and HPV16 or 18 were at greater risk of developing cervical carcinoma than were women infected with only one of the viruses [8, 20].

Different HPV types might infect the female genital tract, and at least 14 HPV types are considered oncogenic or HR HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) [3, 7]. Shin *et al.* [18] reported that the overall prevalence of HPV DNA is 10.4% and that the commonly found HPV DNA types are HPV70, 16, and 33 in women in Busan, South Korea. In our previous study, we found the prevalence of HPV in Korean commercial sex workers (CSWs) to be 47%, with HPV16 and HPV51 being the dominant genotypes [2, 18]. However, the overall prevalence of HPV genotypes in the Korean population is not clearly understood. To develop prophylactic polyvalent vaccines or to estimate effects of drugs or chemical agents against HPV, it is necessary to survey the distribution of HPV genotypes in specific populations.

HPV type distribution in various samples of women from different populations in Korea is not well understood. We investigated the distribution of HPV genotypes in Korean CSWs by using HPV-specific PCR and an HPV oligonucleotide microarray [9, 14]. Additionally, the frequency of HSV1/2 DNA detection and HSV2 seroprevalence values were also determined in the same group.

Between January and December 2003, 188 CSWs who regularly underwent medical examinations were chosen for the HPV/HSV detection trial. Cervical swabs and blood samples were collected from one public health center in Seoul, Korea. The median age of the CSWs was 24 years (range 20–44 years). Cervical cytobrush scrapes were

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suspended in 1 ml of specimen transport medium (BD Bioscience, Franklin Lakes, NJ, U.S.A.) and stored at -20°C until use. All samples were tested for genital HPV DNA and HSV1/2 DNA. Genomic DNA in cervical swabs was extracted by using a Wizard genomic DNA purification kit (Promega, Madison, WI, U.S.A.). The centrifuge tube (15 ml volume) was vortexed to dissociate cells and then centrifuged at 1,000 ×*g* for 3 min. DNA was isolated from cells by lysis followed by protease digestion. RNA was removed by digestion with ribonuclease and DNA was concentrated by ethanol precipitation. The purified DNA samples were stored at -20°C. Purified DNA was used in PCR as templates to amplify target regions. Nested PCR was performed to detect a segment of L1 region with the MY09/MY11 and $GP5^{+}/GP6^{+}$ primer sets [16]. As a control, a 268 bp region of the human β-globin gene was amplified with the GH20/PC04 primer set [17]. HSV nested multiplex PCR was performed as described by Jain *et al.* [10]. In brief, HSV1/2 nested multiplex PCR amplification was performed in magnesium-free reaction buffer supplied with the *Taq* enzyme (Roche, Basel, Switzerland) and primer sets that recognize the glycoprotein B (gpB) region of HSV1 or the glycoprotein G (gpG) region of HSV2.

For HPV genotyping, the HPV L1 gene fragment (about 150 bp) was amplified by using consensus primer sets included in the HPV DNA Chip package (Biomedlab, Seoul, Korea). The HPV DNA Chip contains 22 type-specific probes, 15 for HR genotypes (HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, 69) and 7 for LR genotypes (HPV6, 11, 34, 40, 42, 43, 44). Briefly, HPV DNA was amplified by PCR with specific primers, and β-globin amplifications were also performed as control, which were labeled with Cy5-dUTP (Amersham Biosciences, Uppsala, Sweden). The PCR products were hybridized to the chip at 40°C for 2 h and then the chip was washed for 2 min with 3×SSPE and for 2 min with 1×SSPE. Hybridization signals were visualized by using a DNA Chip Scanner (GSI Lumonics, Scanarray Life, Ottawa, Canada).

All blood samples were tested by using a commercial enzyme-linked immunoabsorbent assay (ELISA) kit

Table 1. Distribution of high-risk and low-risk HPV genotypes in 157 HPV DNA-positive specimens.

Infection	High-risk genotype (n)	High- and low-risk genotype (n)	Low-risk genotype (n)
Single infection	16 (21) 18 (9) 31 (1) 33 (2) 35 (1) 45 (2) 51 (2) 52 (3) 56 (3) 58 (7) 66 (1) 68 (2) 69 (1)		6 (1) 11 (4)
Total	55	0 (0)	5
Multiple infection	16/18 (2) 16/31 (1) 35/45 (1) 16/45 (1) 16/56 (1) 16/33 (1) 16/69 (2) 18/68 (1) 16/18/52 (1) 16/18/56 (2) 16/18/58 (2) 16/39/69 (1) 16/56/58 (1) 51/56/58 (1) 16/18/56/58 (1) 16/18/31/58 (1) 16/18/33/58 (1) 16/18/35/39 (1) 51/52/58/59 (1) 31/58 (1) 33/58 (1) 31/68 (1) 51/69 (1) 52/58 (1) 56/69 (1) 58/68 (1)	11/16 (2) 16/40 (1) 6/18 (2) 6/33 (1) 11/18 (1) 11/39 (1) 40/56 (1) 16/18/6 (1) 16/18/11 (1) 6/33/56/58 (1) 31/34/45/58 (1)	42/43 (1)
Total	30	13	1
Not determined	53		

The HPV genotypes were determined using a HPV DNA Chip. The high-risk HPV genotypes were HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, and 69. The low-risk HPV genotypes were HPV6, 11, 34, 40, 42, 43, and 44. The genotypes for 53 specimens could not be identified by the HPV DNA Chip. Percentage values are indicated in parentheses.

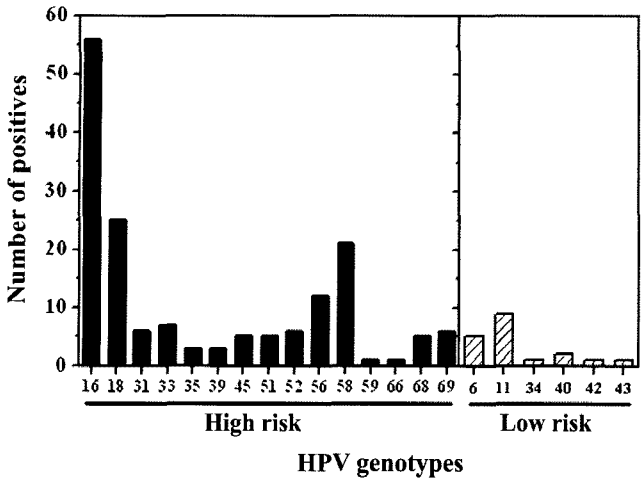

Fig. 1. Prevalence of HPV genotypes in 104 HPV specimens with identified HPV type.
Genomic DNA was extracted from cervical swabs and the HPV L1 gene was amplified by specific primer sets. The PCR products were hybridized onto a HPV DNA Chip containing 22 HPV type-specific oligoprobes. Signals were visualized using a DNA Chip scanner and HPV genotypes were identified. Closed bars: high-risk HPV genotypes. Hatched bars: low-risk HPV genotypes.

(HerpeSelect 2 ELISA IgG, FOCUS Diagnosis, Cypress, CA, U.S.A.) that detects type-specific anti-HSV2 IgG antibody. Fisher's exact test and the Chi-square test were used to investigate the association between HPV and HSV2 prevalences in CSWs. Odds ratios (OR) with 95% confidence intervals (CI) and two tailed *P* values were obtained using the statistical package SAS version 9.1 (SAS Institute Inc., Cary, NC, U.S.A.).

As shown in Table 1, the prevalence of HPV infection in CSWs was 157/188 (83.5%). HPV genotypes were determined in 104 of 157 HPV DNA-positive specimens. The genotypes of 53 specimens could not be determined by the HPV DNA Chip, which is limited to detection of only 22 HPV types. The proportion of HR genotypes (85/157, 54.1%) was much higher than that of LR genotypes (6/157, 3.8%) or that of samples with both high- and low-risk genotypes (13/157, 8.3%). HPV genotypes determined by the HPV DNA Chip were confirmed by direct cycle sequencing (data not shown). The dominant genotypes detected in 188 CSWs were HPV16 (n=56), HPV18 (n=25), HPV56 (n=12), and HPV58 (n=21) in both single and multiple infections (Fig. 1).

HSV DNA was detected in the same group by HSV nested multiplex PCR, and all 188 sera samples were examined for anti-HSV2 IgG antibody [12]. The prevalences of HSV DNA and HSV2 seroprevalence in cervical swab samples were 13.8% and 86.2%, respectively (Table 2). In HSV2 seronegative specimens (n=26), three samples were HSV2 DNA positive. Interestingly, data from 22 (14.0%) of the 188 specimens indicated that HSV2 infections were concurrent with HPV infections. However, this was not statistically significant. The odds ratio was 1.521 and the 95% CI was 0.426–5.433 with P=0.772 when HSV infection frequencies of HPV-positive and HPV-negative groups were compared (Table 3).

We found that the prevalences of HPV infection and HSV seropositivity in 188 CSWs were 83.5% (157/188, including 31 specimens that could not be detected by the HPV DNA Chip because of limitations of detectable HPV genotypes, 22 types) and 86.2% (162/188), respectively. Some 136 (82.4%) of the 165 CSWs shown positive for HSV2 both by DNA assay and ELISA were positive for HPV DNA (data not shown), and 21 (91.3%) of the 23 individuals in the HSV2 seronegative group were also positive for HPV DNA (data not shown). The dominant HR HPV genotypes were HPV16, 18, 56, and 58, and HPV6 and 11 were the most prominent LR HPV genotypes.

The level of HPV DNA positivity found here is higher than the prevalence found in previous studies. The prevalence of HPV in Korean CSWs was 47% (194/417), by HPV DNA Chip microarray [2, 11] using a Hybrid Capture II technique. HPV16 and HPV51 were the dominant genotypes. The prevalence of HPV DNA in cytological smears of patients with cervical neoplasia was 45% (54/120), detected by polymerase chain reaction (PCR) with $GP5^+/6^+$ primer sets [21]. The high prevalence of HPV in our study might reflect the youth of CSWs (20–29 years old) and the sensitive detection method (nested PCR with primers MY09/11 and $GP5^+/GP6^+$). Although HPV DNA of 53 samples was amplified by nested PCR, we could not detect

Table 2. HSV2 prevalence in 188 Korean commercial sex workers.

HSV2 IgG ELISA	PCR detection			
	HSV1	HSV2	ND (%)[a]	Total (%)
Positive (%)	1	22	139 (85.8)	162 (86.2)
Negative (%)		3	23 (14.2)	26 (13.8)
Total	1	25	162	188

Cervical swabs and sera from 188 CSWs were examined for HSV DNA and HSV2 IgG antibody, respectively. HSV type-specific nested multiplex PCR was used for HSV DNA detection in cervical swab material, and HSV2-specific antibody was detected by HSV2 IgG ELISA. Percentage values are indicated in parentheses.
[a]ND: Not detected.

Table 3. Co-infection with HPV DNA and HSV DNA in 188 Korean commercial sex workers.

HPV	HSV2		
	Positive (%)	Negative (%)	Total
Positive	22 (14.0)	135 (86.0)	157
Negative	3 (9.7)	28 (90.3)	31
Total	25	163	188

Odds ratio=1.521 (95% CI, 0.426–5.433), P=0.772. Percentage values are indicated in parentheses.

the genotypes by the DNA microarray method due to limited detactable number of HPV genotypes of the chip. Additionally, we should evaluate the nested PCR protocol using epidemiologically various samples to exclude false positive signals.

Although HSV2 DNA positivity was low in our group (13.3%, 25/188), high HSV2 seroprevalence (86.2%) suggests that most women had been exposed to HSV2 in the past. Higher HSV2 seroprevalences in high-risk populations such as CSWs have been noted in Nigeria [4], Thailand [13], and Bangladesh [5], with values of 59%, 76%, and 63% among CSWs of these countries, respectively. HSV2 seroprevalence was statistically higher than control (25.6%; 95% CI, 23.0–28.2%) in patients with squamous-cell invasive cervical cancer (44.4%; 95% CI, 41.5–47.3%) or adeno-cell invasive or adenosquamous invasive cervical cancer (43.8%; 95% CI, 34.2–53.5%). Moreover, HPV DNA positivity (92.7%) in two carcinoma groups was higher than that of normal subjects (14.7%) [19]. Although our study has low sample numbers and our CSWs did not have cancer, the high prevalence of HPV and HSV2 co-infection suggests that our CSW population might be at high risk for development of cervical cancer.

Although it is difficult to understand the role of HSV2 infection in the etiology of invasive cancer, the presence of HSV2 antibodies in serum with simultaneous HPV DNA infection could lead to a pathological event such as squamous cell dysplasia and, eventually, to invasive cervical cancer. An understanding of the molecular epidemiology of HPV in a particular population might contribute to the design of a more effective HPV vaccine and might permit the evaluation of existing vaccines in Korea.

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