

Distribution and Prevalence of Human Papillomavirus Genotypes in Routine Pap Smear of 2,470 Korean Women Determined by DNA Chip

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Abstract

Purpose: We examined human papillomavirus (HPV) genotype distribution and prevalence from routine Pap smear cases in Korean women using DNA Chip.

Patients and Methods: A total of 2,470 cervical specimens from women attending routine Pap smear cytology screening in local hospitals was subjected to HPV test. HPV detection and genotyping were done using DNA Chip.

Results: HPV DNA was detected in 44.8% of the patients and in 58.7% of the 861 atypical lesions based on the Bethesda system, including 52.6% of 627 atypical squamous cells of undetermined significance (ASCUS), 69.0% of 168 low-grade squamous intraepithelial lesions (LSIL), and 89.4% of 66 high-grade squamous intraepithelial lesions (HSIL) cases. The most frequently found genotypes in all HPV-positive cases were HPV-16, HPV-52, and HPV-58. HPV-16 was the

most prevalent type in within normal limits, ASCUS, and HSIL categories, whereas HPV-51 was most frequently found in LSIL. Multiple infection was identified in about 20% of HPV-positive cases and most of them were that by two different types. HPV-16 was present in the majority of multiple infection cases. A significant decrease in the percentage of multiple infection was observed in HSIL cases compared with ASCUS and LSIL.

Conclusions: The distribution of HPV genotypes in Korean women was revealed to have differences to that of other regions, showing higher frequencies of HPV-52, HPV-58, and HPV-51. HSIL cases were mostly infected by sole HPV-16 whereas LSIL that by various HPV types, suggesting a certain type may become dominant over others as the disease progresses. (Cancer Epidemiol Biomarkers Prev 2004;13(12):2153-6)

Introduction

It has been commonly accepted that persistent infection with certain human papillomavirus (HPV) genotypes is the strongest etiologic factor for the development of cervical cancer. These HPV genotypes detected in more than 90% of all cervical cancer cases are now classified as human carcinogen. Because the distribution and prevalence of HPV vary by geographic region and the immunity conferred by vaccines is type-specific, the need for HPV genotyping in routine screening population is increasingly recognized. HPV infection is known to be etiologic for the development of cervical cancers and their pathologic precursors, as infection by particular types of HPV increases the risk of developing invasive disease (1-3). High-risk types are considered oncogenic, as they are found in more than 90% of the cervical tissues of patients with cervical carcinoma or cervical intraepithelial neoplasia. Low-risk types, on the other hand, only cause benign lesions (4, 5).

HPV-16 is the most prevalent type worldwide. However, the second most prevalent one is HPV-18 in Western countries, whereas it is HPV-58 in Asia (6). HPV infection by multiple genotypes has been reported to occur in 10% to 20% of HPV-positive cases and to show dependence on HPV type, age of the patient, and the presence of cytologic abnormalities (7). These epidemiologic findings in combination with follow-up studies often provide valuable information on the role of certain HPV types in cervical cancer development.

HPV testing has been used as an adjunct to Pap smear cytology test in the diagnosis of cervical cancers to improve screening sensitivity and negative predictive value (8-10). In fact, HPV testing is now recommended for patients with cytologic abnormalities based on the new American College of Obstetricians and Gynecologists guidelines. In addition, the importance of HPV genotyping in diagnostic practices is increasingly recognized in distinguishing high-risk from low-risk infections as well as in the development of cancer intervention strategies such as vaccine preparation (11). Based on these facts, genotyping of HPV in clinical settings is regarded not only as an important diagnostic tool for cervical cancer but also as means for providing valuable information necessary for its prevention and treatment.

In this study, we examined HPV genotype distribution and prevalence from routine Pap smear cases in Korean women using commercially available HPV DNA Chip

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(Biomedlab Co., Seoul, Korea). Cytologic diagnosis based on the new Bethesda system and the presence and distribution of 22 clinically significant HPVs were compared to investigate the profile of HPV infection in Korean Women and to speculate its influence on disease progression.

Material and Methods

Specimen Collection and Pretreatment. Cervical swab samples of patients who visited regional hospitals in Korea for Pap smear between October 2002 and May 2003 were used in the study. Specimens for HPV test obtained by gentle swabbing with a cytobrush sampler after Pap smear sampling were placed in a test tube containing 1 mL of sterile PBS solution. The mixture was vortexed to dissociate cells and centrifuged at $10,786 \times g$ for 5 minutes. After removing the supernatant, DNA was extracted by a commercial AccuPrep Genomic DNA Extraction Kit (Bioneer Co., Seoul, Korea) following the manufacturer's recommendations. The result of Pap smear was followed up in which cytologic diagnosis was made with the new Bethesda system as follows; within normal limits (WNL), atypia of squamous cells of undetermined significance (ASCUS), low-grade squamous intraepithelial lesions (LSIL), and high-grade squamous intraepithelial lesions (HSIL).

Human Papillomavirus Detection and Genotyping. Specimens of 2,470 randomly selected swabs were analyzed by commercial HPV DNA Chip (Biomedlab) for detection and genotyping of 22 HPV types (15 high risk: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, 69 and 7 low risk: 6, 11, 34, 40, 43, 44) according to the manufacturer's protocol. Briefly, the target DNA was amplified by PCR using the provided primers, hpv1/2 for HPV DNA and bg1/2 for human β -globin. During the PCR, Cy5-dUTP (NEN Life Science, Inc., Boston, MA) was used to randomly incorporate Cy5 labeling to the product. The products were hybridized onto the chip at 40°C for 2 hours. The chip was washed serially with $3 \times$ saline-sodium phosphate-EDTA for 2 minutes and $1 \times$ saline-sodium phosphate-EDTA for 2 minutes and air-dried at room temperature. Hybridization signals were visualized by fluorescence scanning in a commercial DNA chip scanner (GenePix Pro3.0, Axon Instruments, Inc., Union City, CA).

Statistical Analysis. Statistical analyses were done to identify factors significantly associated with the presence of HPV. Statistical tests were done by χ^2 test using SAS version 8.0 for Windows (SAS Institute, Inc., Cary, NC). $P \leq 0.05$ was considered statistically significant.

Results

Pap smear result of 2,470 patients were found to include 1,609 WNL, 627 ASCUS, 168 LSIL, and 66 HSIL. Table 1 summarizes the result of HPV DNA detection by DNA chip in each cytologic category. HPV DNA was found in 44.8% (1,106 of 2,470) of the total cases and in 58.7% (505 of 861) of the atypical lesions, 330 (52.6%) of 627 ASCUS, 116 (69.0%) of 168 LSIL, and 59 (89.4%) of 66 HSIL,

Table 1. HPV positivity by HPV DNA Chip in each cytologic category

Cytologic diagnosis	HPV DNA Chip result		Total no.*
	No. negative (%)	No. positive (%)	
WNL	1,008 (62.6)	601 (37.4)	1,609
ASCUS	297 (47.4)	330 (52.6)	627
LSIL	52 (31.0)	116 (69.0)	168
HSIL	7 (10.6)	59 (89.4)	66
Total no. (%)	1,364 (55.2)	1,106 (44.8)	2,470 (100)

* $P < 0.0001$ (χ^2 test).

classified according to the Bethesda system ($P < 0.0001$). The prevalence of HPV genotypes in relation with cytologic diagnosis is summarized in Table 2. HPV-16 detected in 204 cases was significantly the most common type ($P < 0.0001$) in all HPV DNA-positive patients followed by HPV-51 (88 cases; $P < 0.0001$), HPV-52 (110 cases; $P = 0.0077$), and HPV-58 (110 cases; $P = 0.001$) in 846 high-risk type patients including multiple infection cases. HPV-51 was the most common type in LSIL detected in 10.7% (18 of 168) of the cases. There was no statistically significant difference between the cytologic groups with respect to the distribution and prevalence of HPV types.

Multiple infections were identified in about 20% of the HPV-positive cases. The detection frequency of single and multiple HPV infection with respect to cytologic diagnosis is shown in Fig. 1. In 1,106 HPV-positive samples, single infections were detected in 62.8%, infection by two different types were detected in 16.4%, three types in 3.0%, four types in 1.0%, and five types in 0.5% of the total cases. Infections by HPV types not included on the chip ("other types") were considered single infection and were detected in 16.3%. The frequency of multiple HPV infection was higher in LSIL than in other lesions (25.9% in LSIL compared with 13.6% in HSIL; $P < 0.0001$). Other types were found more intensively in WNL than in the other cytologic categories (113 cases, 18.8% of 601 WNL, data not shown).

The occurrence of HPV types with respect to cytologic diagnosis was determined by calculating the percentage of each HPV from the total HPV occurrence in each category (Fig. 2). The total HPV occurrence was determined to be 1,410 including 780 in WNL, 405 in ASCUS, 155 in LSIL, and 70 in HSIL. The most prevalent HPV genotypes were HPV-16 (14.5%) followed by HPV-52 and HPV-58 (7.8% each). HPV-51 was present in 6.2%, HPV-40 in 4.7%, HPV-18 in 4.3%, and HPV-35 in 4.3% of the total occurrence. The correlation between high-risk HPV presence and HSIL was significantly higher ($P < 0.0001$) compared with LSIL in which various HPV types were dispersed. The frequency of HPV-16 was observed significantly higher in HSIL than in the other cytologic groups (32.9% in HSIL compared with 9.7% in LSIL; $P < 0.0001$). The second most frequent types were HPV-58 ($P = 0.001$) and HPV-52 ($P = 0.0077$). HPV-51 was detected the higher in LSIL than in HSIL (11.6% versus 4.3%, $P < 0.0001$). High-risk type HPV-45, HPV-66, HPV-68, and low-risk type HPV-6, HPV-42, HPV-43 were not found in HSIL lesions in this study. The frequency of other

Table 2. Prevalence of HPV genotypes based on cytologic diagnosis

HPV type detected	Cytologic diagnosis				
	WNL, <i>n</i> = 1,609 (%)	ASCUS, <i>n</i> = 627 (%)	LSIL, <i>n</i> = 168 (%)	HSIL, <i>n</i> = 66 (%)	Total, <i>n</i> = 2,470 (%)
High-risk type* [†]	448 (27.8)	254 (40.5)	89 (53.0)	55 (83.3)	846 (34.3)
HPV-16	101 (6.3)	65 (10.4)	15 (8.9)	23 (34.9)	204 (8.3)
HPV-51	47 (2.9)	20 (3.2)	18 (10.7)	3 (4.6)	88 (3.6)
HPV-52	55 (3.4)	39 (6.2)	12 (7.1)	4 (6.1)	110 (4.5)
HPV-58	60 (3.7)	31 (4.9)	10 (6.0)	9 (13.6)	110 (4.5)
Low-risk type [†]	87 (5.4)	51 (8.1)	19 (11.3)	4 (6.1)	161 (6.5)
Other type [‡]	113 (7.0)	44 (7.0)	21 (12.5)	2 (3.0)	180 (7.3)

* $P < 0.0001$ (χ^2 test).

[†]No. patients including multiple infection of each type.

[‡]HPV types other than the 22 placed on the DNA Chip.

types, HPV types other than the 22 placed on the chip, determined by positive PCR and negative chip result was 12.8% in all HPV occurrences (WNL, 14.5%; ASCUS, 10.9%; LSIL, 13.5%; HSIL, 2.9%; $P = 0.033$).

Discussion

Because the recognition of HPV as the major etiologic factor for cervical cancers, HPV testing has been employed as the first confirmation method done when changes in cellular morphology was observed in the Pap smear (12-14). Abnormalities of ASCUS or more based on the Bethesda system are recommended for HPV tests, which greatly enhances the screening efficiency for cervical cancers.

As the volume of HPV test increases, the clinical and epidemiologic studies including those on the carcinogenic role of HPV as well as genotype distribution and prevalence have been advanced. These research outcomes are valuable for the development of cancer intervention strategies and therapeutics. Especially for vaccine development, viral DNA detection and typing in relation with screening for low- or high-grade intra-epithelial lesions should be included in determining the vaccine valency and efficacy (15, 16). Recent molecular epidemiologic evidence has lead to the reestablishment of the high- or low-risk types, in which many additional

high-risk types other than the traditional ones like HPV-16 have been defined (17). Also in up to 20% of HPV-positive cases, multiple genotypes are found, which results in a higher HPV occurrence at a population level (18). In determining the clinical role of HPV types as well as in the development of means for treatment and prevention, the HPV molecular epidemic has to be effectively assessed in a population-specific manner. To accomplish this, a sensitive and reliable technique for HPV detection and typing in either single or multiple infections that can be easily adapted in routine screening is required.

Recently, many reports on the HPV oligonucleotide microarray (HPV DNA Chip) have been published describing its diagnostic potential and effectiveness (19-21). The method was reported to be highly specific and sensitive showing the detection limit of 18fg HPV DNA (9). This study was conducted by using the HPV DNA Chip capable of detecting infection by 22 HPV types. HPV infection profile and type distribution in Korea was determined based on the examination of 2,470 routine Pap smear cases.

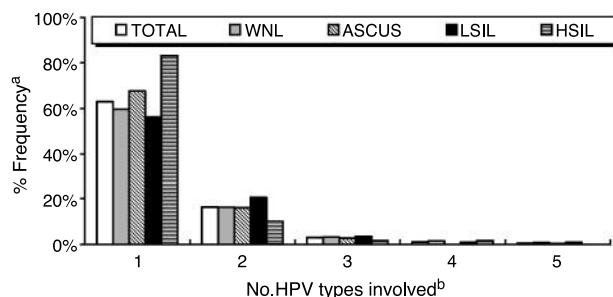


Figure 1. The frequency of single and multiple HPV infection with respect to cytologic diagnosis. ^aPercentage calculated based on 1,106 HPV-positive cases. ^bNumber of HPV types involved in one infection (1, single infection; ≥ 2 , multiple infection).

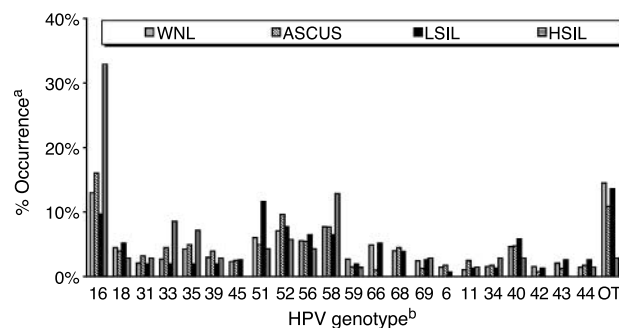


Figure 2. HPV type occurrence in the cytological categories. ^aPercentage calculated based on the HPV occurrence in each cytological category (780 in WNL, 405 in ASCUS, 155 in LSIL, and 70 in HSIL). Types in multiple infection cases were individually counted. Other types denote HPV types not included in the current version of HPV DNA Chip. Other types were determined when PCR-positive cases showed no signal on the chip. ^bHigh-risk HPV types are on the left (16-19) and the low-risk types on the right (6-44).

Thus, we identified three major HPV genotypes in the study population, HPV-16 (14.5%), HPV-52 (7.8%), and HPV-58 (7.8%). Other prevalent types were HPV-51 (6.2%), HPV-40 (4.7%), HPV-18 (4.3%), and HPV-35 (4.3%). These findings show differences from those of other reports in terms of the percentages of each type. The prevalence of HPV-51 and HPV-52 were noticeably higher and that of HPV-18 lower. HPV-6 and HPV-11 have been reported to be restricted to exophytic condyloma and not associated with HSIL (20). In this study, HPV-11 was found in HSIL although not prevalent. HPV-6 was not found in HSIL. Several other types, including high-risk type HPV-45, HPV-66, and HPV-68 and low-risk type HPV-42 and HPV-43, were also not found in HSIL. HPV-51 was the most prevalent type in LSIL. The distribution of HPV-51 with respect to the cytologic categories was similar to that of HPV-18 showing lower frequency in HSIL than LSIL. The presence of HPV-18 is often considered as an indication of worse clinical outcome and recurrence possibility (22, 23). The frequency of multiple infection was about 20% of all HPV-positive cases, in which infection by two different types were the most prevalent. HPV-16 was found in most multiple infections. It was interesting to note that the percentage of multiple infection was significantly decreased from LSIL to HSIL. In HSIL cases, HPV-16 was the most common type. Based on these observations, it is likely that a single potent type may become dominant over others as the disease progresses.

In conclusion, HPV infection in Korean women examined with routine Pap smear cases showed difference in type distribution and prevalence from those reported elsewhere. However, HPV-16 was still the most prevalent and considered the most potent carcinogen. Similarly, it was found that the frequency of HPV-52 and HPV-58 is increasing in cervical malignancies as previously described in other studies. The higher prevalence of HPV-51 and its clinical role as well as the clinical implication of multiple infections need to be addressed through further studies.

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