

Prevalence and Genotype Distribution of Cervical Human Papillomavirus DNA in Korean Women: A Multicenter Study

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*MyGene Co. (Seoul, Korea) provided equipments and technical support for HPV DNA chip testing in each laboratory and donated the HPV DNA chip kits.

Background : DNA prevalence and type distribution of human papillomavirus (HPV) varies geographically. We investigated HPV prevalence and type distribution in Korean women using the MyHPV DNA chip testing. **Methods :** A total of 2,368 women from five regions of the country underwent Pap smear examination and MyHPV chip testing. **Results :** Overall HPV positivity was 15.8% and 78.4% in women with normal and abnormal cytology, respectively. High-risk HPV infection was strongly correlated with cytological atypia. In women with abnormal cytology, the five most common HPV types were 16, 58, 18, 52, and 56/53, and HPV16 was significantly the most common type in most geographical regions. After HPV16, HPV58, and 52 were the next most frequently detected types. Women with normal cytology, in contrast, showed heterogeneity in HPV type distribution. High-grade intraepithelial lesions infected with HPV16, 18, 31 or 45 are more likely to progress to carcinoma. **Conclusions :** The HPV chip test can provide useful data regarding HPV positivity and type. The most common HPV type in Korean women with abnormal cytology is HPV16, with HPV58 and 52 being frequently present. Our data may have important implications for vaccination programs and the development of cervical screening.

Key Words : Uterine cervix; Human papillomavirus; DNA chips; Papanicolaou test; South Korea

Cytological screening using Papanicolaou (Pap)-stained smears for cervical cancer has proven to be a simple and effective method for reducing cervical cancer incidence and mortality during the last century. Even so, screening with Pap testing alone is unlikely to detect all cervical neoplasias. Molecular and epidemiologic studies have proven that virtually all cervical cancers and their precursor lesions are causally related to infection by HPV.¹ The HPV family includes over 100 viral types, of which more than 40 infect the genital tract. These viruses can be divided into three groups: "high-risk (HR)" viruses associated with a high relative risk of cervical cancer, "low-risk (LR)" viruses associated with a low risk of cervical cancer, and "intermediate-risk (IR)" viruses occasionally associated with cervical cancer.²

In a prospective follow-up study, while the cumulative incidence of cervical intraepithelial neoplasia 3 (CIN3) was 4.4% among HR-HPV positive women followed-up for up to 45 months, it was only 0.24% among HR-HPV negative women.³ HR-HPV DNA testing has potential clinical applications, especially in women with atypical squamous cells of undetermined significance (ASC-US),⁴ postmenopausal women with low-grade squamous intraepithelial lesion (LSIL),⁵ follow-up of adolescents with LSIL,⁶ post-colposcopy follow-up,⁷ and post-treatment follow-up of high-grade CIN.⁸ The American Cancer Society has recommended HPV DNA testing in conjunction with cytology as a screening option for women aged 30 years and older,⁶ and has developed guidelines for use of the prophylactic HPV vaccine in the prevention of CIN and cervical cancer.⁹ Considering these findings and recommendations, HR-HPV testing could have important roles in the management of women with abnormal cytology, primary cervical cancer screening, and the development of HPV vaccines.

There is great regional variability in HPV DNA prevalence and genotype distribution worldwide.^{10,11} In a meta-analysis of women with normal cytology, the prevalence of HPV was higher in less developed countries (15.5%) than in more developed countries (10.0%), and higher in younger as compared to older women.¹⁰ On the other hand, among women with invasive cervical cancer, the overall detected HPV prevalence and HPV type distribution appears to vary little between geographical regions.¹¹ In most regions, after HPV16 and 18, HPV 45, 31, and 33 are the most prevalent in invasive squamous cell carcinomas. In Asia, however, HPV58 and 52 are found more common than HPV45, 31, and 33. Further research on HPV prevalence and HPV type distribution would not only affect the development of cancer-screening HPV tests, but could also help predict the effect of HPV vaccines.

At present, there are two testing systems for HPC detection

in widespread clinical use: hybrid capture technology and PCR-based methods. The MyHPV Chip kit (MyGene, Seoul, Korea), which is based on a combination of HPV DNA chip technology and DNA hybridization, has been developed and approved for clinical application by the Korean Food and Drug Administration. The chip kit tests for 24 different HPV genomic sequences: HPV 6, 11, 16, 18, 31, 33, 34, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 56, 58, 59, 66, 68, and 70.¹²

Presently, we used HPV DNA chip testing to gather information on HPV prevalence and HPV-type distribution according to region and cytology from a large sample of South Korean women.

MATERIALS AND METHODS

Study population and specimen collection

Source materials were gathered from 21 South Korea hospitals located in Seoul, Incheon, Gyeonggi, Daejeon (Chungnam), Daegu (Gyeongbuk), Busan, Jeonbuk, and Gwangju from October 2005 to August 2006. These hospitals were divided into five regions: capital region (Seoul, Incheon and Gyeonggi), Daejeon, Daegu, Busan and Honam (Jeonbuk and Gwangju). The size of the participant sample in each region was based roughly on local population numbers. The study process in each hospital was performed under the same protocol. After routine medical examination by gynecologists, HPV test samples and Pap smears were obtained from women who had no specific gynecological history within the last 3 months. All participating women gave written informed consent for this study. This study was approved by the ethical committees at all of participating hospitals that had such committees.

Cytology test

Conventional Pap smears or liquid-based cytological smears obtained using standard procedures were interpreted by the pathologists in each laboratory for the purposes of clinical use. Cytological diagnoses were classified by 2001 Bethesda System terminology. To minimize bias from cytological-interpretation error, we selected hospitals that have been inspected and accredited by the quality management programs of the Korean Society for Cytopathology. For quality assurance to detect errors of interpretation, Pap smears were rescreened with 10% random selection of slides by the regional study groups.

HPV test

Participating laboratories had an approximately 1-year preparatory period to familiarize themselves with the MyHPV DNA chip test before HPV testing started for study purposes. HPV tests in each laboratory were performed by the technicians in charge of HPV testing under the supervision of both the pathologists and the MyGene laboratory. The risk levels of the 24 HPV types in the MyHPV Chip kit were classified into HR-HPV (HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68), IR-HPV (HPV53 and 66), or LR-HPV (HPV6, 11, 34, 40, 42, 43, 44, 54, and 70).²

Samples for HPV testing were collected separately from Pap smears using endocervical brushes supplied with the MyHPV Chip kit. Briefly, DNA was isolated using an isolation kit (MyGene) and nested PCR was performed for gene amplification. For the first PCR, a mixture of two set primers, MY09 (5'-CGT CCM ARR GGA WAC TGA TC-3', 20 pmol) and MY11 (5'-GCM CAG GGW CAT AAY AAT GG-3', 20 pmol), were used. For the second PCR, HPV primers GP5+ (5'-TTT GTT ACT GTG GTA GAT ACT AC-3', 20 pmol) and GP6+ (5'-GAA AAA TAA ACT GTA AAT CAT ATT C-3', 20 pmol) were used. β -globin PCR amplification was used as an internal control. PCR products were subjected to electrophoresis in a 3% agarose gel and 250 and 150 bp PCR products were extracted after the first and the second PCR, respectively. Ten microliters of the second PCR product was denatured for 5 min at 95 °C and left in ice water for 5 min. The product was then mixed with hybridization buffer (MyGene). This mixture was spotted on the DNA chip and subjected to a hybridization reaction for 1 h in a hybridization chamber (Vision Scientific, Seoul, Korea) at 43 °C. After hybridization, the product was rinsed with buffer I (2X standard saline citrate [SSC], 0.1% sodium dodecyl sulfate [SDS]), buffer II (0.2X SSC) and buffer III (0.1X SSC) twice, twice and once, respectively, for 5 min. Hybridized HPV DNA was identified using a model G4000 scanner (GSI Lumonics, Billerica, MA, USA). A pair of oligonucleotide probes for each of the 24 HPV types was attached to each DNA chip. When a pair of specific probes fluoresced, the assay was determined to be positive for that HPV type.

Statistical analysis

For comparison of prevalence rates, HPV type-specific prevalence and heterogeneity of HPV type, statistical analysis was performed using the chi-square test with Statistical Package Service

Solution software (SPSS for Windows, standard version 14.0; SPSS, Chicago, IL, USA). P-values <0.05 were considered statistically significant and 95% confidence intervals were calculated.

RESULTS

Study population

A total of 2,368 women from five regions in South Korea participated in this multicenter study. The largest sample consisted of cases from the capital region including Seoul, Incheon and Gyeonggi (1,225, 51.7%), followed by cases from Busan (391, 16.5%), Honam (350, 14.8%), Daegu (268, 11.4%) and Daejeon (134, 5.7%). According to the cytological interpretations, 1,960 participants had normal cytology and 408 had abnormal cytology. Abnormal cytological findings were composed of 171 atypical squamous cells (ASC), six atypical glandular cells (AGC), 86 LSIL, 101 high-grade squamous intraepithelial lesions (HSIL), one adenocarcinoma in situ (AIS), and 43 invasive carcinomas (IC). The 171 ASC included 149 ASC-US and 22 ASC cannot exclude HSIL (ASC-H), and the 43 IC included 38 squamous cell carcinomas (SCC) and five adenocarcinomas. The overall mean age of all 2,368 women was 45.7 years (range 20-94 years); women with normal compared to abnormal cytology had mean ages of 45.5 and 47.0 years respectively.

Overall HPV prevalence

Overall, HPV DNA was detected in 629 (26.6%) of the 2,368 women. HPV frequencies in women with normal compared to abnormal cytology were significantly different ($p=0.00$). Among women with normal cytology, 15.8% (309 of 1,960) were HPV-positive compared to 78.4% (320 of 408) of women with abnormal cytology (Table 1). The prevalence of overall infection with single, multiple, and other types (presence of amplified HPV-PCR products without detection of specific HPV genotypes by the HPV DNA chip test) of HPV were 399 (63.4%), 92 (14.6%) and 138 (21.9%), respectively, among the 629 HPV-positive women. Within the normal and abnormal cytology groups, there were no significant differences in prevalence for infections with single, multiple, and other types of HPV ($p>0.05$), although women with abnormal cytology tended to be infected more frequently with multiple types (11.7% vs 17.5% for normal cytology).

HPV prevalence by region for women with normal cytology

The samples obtained from the 1,960 women with normal cytology were composed of 1,026 (52.3%) cases from the capital region, 377 (19.2%) cases from Busan, 242 (12.3%) cases from Honam, 214 (10.9%) cases from Daegu, and 101 (5.2%) cases from Daejeon. Comparing overall HPV positivity by region, positivity rates were similar for all five areas, ranging from 15.1% in Busan to 17.8% in Daejeon (Table 2). HPV infection rates were varied widely by hospital (9.7-22.8%; data not shown). Additionally, HR-HPV frequencies in women with normal cytology differed by region. The average prevalence of HR-HPV infection was 8.0%, with 8.2-10.2% infection rates in the high-frequency areas (Daegu, Honam and the capital region), and 5.0-6.6% infection rates in the low-frequency areas (Daejeon and Busan).

Table 1. HPV infection in women with normal and abnormal cytology

Cytology	HPV+ (%)	HPV infection pattern (%)		
		Single	Multiple	Other
Total (n=2,368)	629 (26.6)	399 (63.4)	92 (14.6)	138 (21.9)
Normal (n=1,960)	309 (15.8)	201 (65.0)	36 (11.7)	72 (23.3)
Abnormal* (n=408)	320 (78.4)	198 (61.9)	56 (17.5)	66 (20.6)

*atypical squamous or glandular cells and more.

HPV prevalence in women with abnormal cytology

Overall HPV prevalence (78.4%) in women with abnormal cytology was correlated with cytological interpretation ($p=0.00$) (Table 3). The HPV-positivity rate was higher in women with HSIL+/AIS+ (91.7%, 133 of 145) and LSIL (90.7%, 78 of 86) compared to women with ASC-H/AGC (67.9%, 19 of 28) and ASC-US (60.4%, 90 of 149) ($p=0.00$). In terms of HPV-risk frequency, the HR-HPV infection rate was higher than the LR-HPV rate for all cytological diagnoses. Even among women with normal cytology, HR-HPV infections accounted for approximately 51% (157 of 309) of infections in the HPV-positive women. The proportion of HR-HPV infection was significantly higher in women with HSIL+/AIS+ (73.8%, 107 of 145) compared to LSIL (53.5%, 46 of 86) ($p=0.00$), while relatively higher in women with ASC-H/AGC (46.4%, 13 of 28) compared to ASC-US (34.2%, 51 of 149) ($p>0.05$). Region-specific HR-HPV prevalence was variable and ranged from 39.2-71.3% in women with abnormal cytology: 25.0-60.0% in ASC-US, 35.7-80.0% in ASC-H/AGC, 40.4-87.5% in LSIL, and 65.6-80.0% in HSIL+/AIS+ (data not shown).

HPV prevalence according to age

The participants were stratified into three age categories: young

Table 2. HPV prevalence according to region for normal cytology

	Capital region (%)	Daejeon (%)	Daegu (%)	Busan (%)	Honam (%)	Total (%)
Total	1,026 (100)	101 (100)	214 (100)	377 (100)	242 (100)	1,960 (100)
HPV (-)	869 (84.7)	83 (82.2)	177 (82.7)	320 (84.9)	202 (83.5)	1,651 (84.2)
HPV (+)	157 (15.3)	18 (17.8)	37 (17.3)	57 (15.1)	40 (16.5)	309 (15.8)
HR-	84 (8.2)	5 (5.0)	22 (10.2)	25 (6.6)	21 (8.7)	157 (8.0)
NonHR-	39 (3.8)	6 (5.9)	7 (3.3)	19 (5.0)	9 (3.7)	80 (4.1)
Other-	34 (3.3)	7 (6.9)	8 (3.7)	13 (3.4)	10 (4.1)	72 (3.7)

HR-, high risk HPV; NonHR-, non-high risk HPV.

Table 3. HPV prevalence according to cytological diagnosis

	Normal (%)	Abnormal cytology (%)				
		Abnormal	ASC-US	ASC-H/AGC	LSIL	HSIL+/AIS+
Total	1,960 (100)	408 (100)	149 (100)	28 (100)	86 (100)	145 (100)
HPV (-)	1,651 (84.2)	88 (21.6)	59 (39.6)	9 (32.1)	8 (9.3)	12 (8.3)
HPV (+)	309 (15.8)	320 (78.4)	90 (60.4)	19 (67.9)	78 (90.7)	133 (91.7)
HR-	157 (8.0)	217 (53.2)	51 (34.2)	13 (46.4)	46 (53.5)	107 (73.8)
NonHR-	80 (4.1)	37 (9.1)	16 (10.7)	1 (3.6)	13 (15.1)	7 (4.8)
Other-	72 (3.7)	66 (16.2)	23 (15.4)	5 (17.9)	19 (22.1)	19 (13.1)

HR-, high risk HPV; NonHR-, non-high risk HPV; ASC-US, atypical squamous cells, undetermined significance; ASC-H, atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion; AGC, atypical glandular cells; LSIL, low-grade squamous intraepithelial lesion; HSIL+, high-grade squamous intraepithelial lesion and more; AIS+, adenocarcinoma in situ and more.

group (women <30 year-of-age, n=143), women between 30-59-years-of-age (n=1,945); and elderly group (≥ 60 years-of-age, n=280). Table 4 shows the frequency of HPV infection according to these age categories. The women aged <30 years displayed the highest frequency of HPV infection among women with both normal (21.8%) and abnormal (93.3%) cytology. The second highest frequency was found in women ≥ 60 -years-of-age for both women with normal (19.3%) and abnormal (82.2%) cytology. The frequency of HR-HPV infection stratified by age categories also had the same pattern.

HPV type-specific prevalence by cytological interpretation

Women infected with multiple types of HPV were counted more than once, because each type in a multiple-type infection was considered individually to be a constituent type. Among the 491 HPV-positive women with single or multiple types (excepting other-type infections), 237 women with normal cytology had a total of 279 HPV infections, and 254 women with abnormal cytology had a total of 323 HPV infections. Table 5 shows type-specific HPV prevalence according to cytological interpretation. The categories ASC-US and ASC-H/AGC were combined into

Table 4. HPV prevalence according to age

Age (years)	Normal cytology (%)			Abnormal cytology (%)		
	No.	HPV (+)	HR-HPV	No.	HPV (+)	HR-HPV
<30 (n=143)	110	24 (21.8)	16 (14.5)	33	31 (93.9)	21 (63.6)
30-59 (n=1,945)	1,643	245 (14.9)	121 (7.7)	302	229 (75.8)	151 (50.0)
≥ 60 (n=280)	207	40 (19.3)	20 (9.7)	73	60 (82.2)	45 (61.6)

HR-HPV, high-risk HPV.

Table 5. Type-specific HPV prevalence in women with normal and abnormal cytology

	Type	Normal (%)	Abnormal cytology (%)					Total
			ASC/AGC	LSIL	HSIL/AIS	IC	HSIL+/AIS+	
HR	16	24 (6.8)	15 (11.5)	12 (12.4)	37 (31.4)	16 (37.2)	53 (32.9)	80 (20.6)
	18	40 (11.4)	6 (4.6)	10 (10.3)	9 (7.6)	6 (14.0)	15 (9.3)	31 (8.0)
	31	10 (2.8)	9 (6.9)	1 (1.0)	2 (1.7)	4 (9.3)	6 (3.7)	16 (4.1)
	33	5 (1.4)	4 (3.1)	3 (3.1)	8 (6.8)	1 (2.3)	9 (5.6)	16 (4.1)
	35	3 (0.9)	4 (3.1)	2 (2.1)	5 (4.2)		5 (3.1)	11 (2.8)
	39	6 (1.7)	2 (1.5)	2 (2.1)	1 (0.8)		1 (0.6)	5 (1.3)
	45	5 (1.4)	2 (1.5)	2 (2.1)	1 (0.8)	2 (4.6)	3 (1.9)	7 (1.8)
	51	1 (0.3)	2 (1.5)	3 (3.1)				5 (1.3)
	52	22 (6.3)	8 (6.1)	2 (2.1)	7 (5.9)	2 (4.6)	9 (5.6)	19 (4.9)
	56	14 (4.0)	6 (4.6)	9 (9.3)	3 (2.5)		3 (1.9)	18 (4.6)
	58	22 (6.3)	16 (12.2)	6 (6.2)	16 (13.6)	4 (9.3)	20 (12.4)	42 (10.8)
	59	4 (1.1)	1 (0.8)	1 (1.0)				2 (0.5)
	68	13 (3.7)	3 (2.3)	3 (3.1)	1 (0.8)	1 (2.3)	2 (1.2)	8 (2.1)
	IR	53	31 (8.8)	7 (5.3)	8 (8.2)	2 (1.7)	1 (2.3)	3 (1.9)
66		7 (2.0)	7 (5.3)	4 (4.1)	4 (3.4)	1 (2.3)	5 (3.1)	16 (4.1)
LR	6	15 (4.3)	3 (2.3)	1 (1.0)	2 (1.7)	1 (2.3)	3 (1.9)	7 (1.8)
	11	3 (0.9)	1 (0.8)	1 (1.0)				2 (0.5)
	34			1 (1.0)				1 (0.3)
	40	5 (1.4)						
	42	1 (0.3)		1 (1.0)				1 (0.3)
	43	1 (0.3)						
	44	1 (0.3)						
	54	17 (4.8)	4 (3.1)	1 (1.0)	3 (2.5)	1 (2.3)	4 (2.5)	9 (2.3)
	70	29 (8.3)	3 (2.3)	5 (5.2)	1 (0.8)		1 (0.6)	9 (2.3)
Other	72 (20.5)	28 (21.4)	19 (19.6)	16 (13.6)	3 (7.0)	19 (11.8)	66 (17.0)	
HPV (+)	351 (100)	131 (100)	97 (100)	118 (100)	43 (100)	161 (100)	389 (100)	

HR, high risk; IR, intermediate risk; LR, low risk; ASC, atypical squamous cells; AGC, atypical glandular cells; LSIL, low-grade squamous intraepithelial lesion; HSIL, high-grade squamous intraepithelial lesion; AIS, adenocarcinoma in situ; IC, invasive carcinoma.

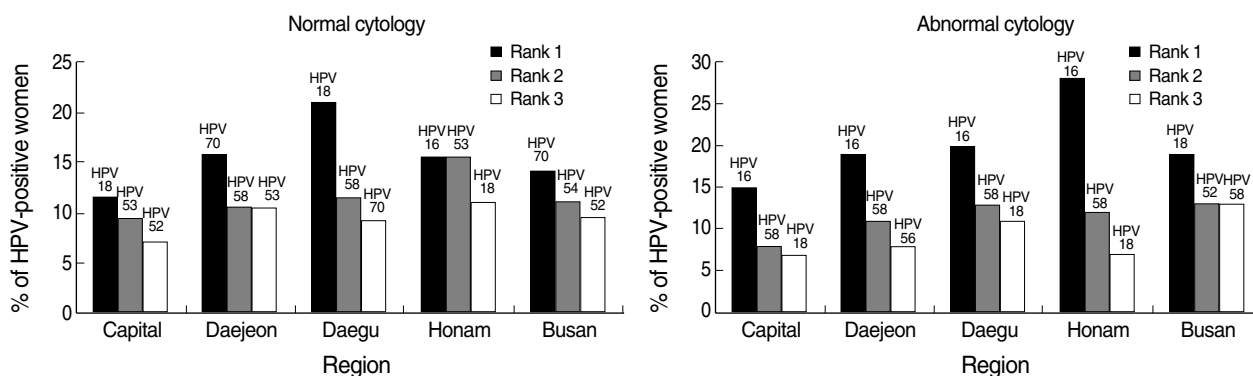


Fig. 1. Regional variation in the three most common HPV types among women with normal and abnormal cytology.

one category, ASC/AGC, because of the very small numbers of ASC-H/AGC study cases. HPV type-specific prevalence was significantly different between women with normal and abnormal cytology ($p=0.00$). In women with normal cytology, the five most common types were HPV18, 53, 70, 16, and 52 or 58, in descending order of frequency. In women with abnormal cytology, HPV16, 58, 18, 52, and 56 or 53 were the five most common types in descending order. Among women with HSIL+/AIS+, the most common type was HPV16, followed by 58, 18, 33, and 52. The HPV16-positivity rate was correlated with the degree of cytological atypia ($p=0.00$). The five most common HPV types were found in variable frequencies according to cytological interpretation, from 42% in women with normal cytology to 70% in women with IC.

HPV type-specific prevalence by region

Fig. 1 displays the three most common HPV types by region in women with normal and abnormal cytology. Women with normal cytology had a more heterogeneous distribution of HPV types than those with abnormal cytology, but this difference was statistically insignificant ($p>0.05$). In women with normal cytology, six different types of HPV (18, 70, 53, 58, 54, and 52) were identified as among the three most common types in each of the five regions. The most common HPV type was HPV 18 in two areas (capital area and Daegu), HPV70 in two areas (Daejeon and Busan), and HPV16 or 53 together in one area (Honam). In women with abnormal cytology, five types of HPV (16, 58, 18, 52, and 56) were among the three most common types in each of the five regions. HPV16 was identified as the most common type in all areas with the exception of Busan, in where the most common type was HPV18. Women with HSIL+/AIS+ were also infected most commonly with HPV16 in each region, again with the exception of Busan.

DISCUSSION

HPV prevalence in women with normal cytology

In this study, the overall prevalence of HPV DNA was 15.8% in women with normal cytology. This finding is similar to that of a large worldwide meta-analysis of women with normal cervical cytology.¹⁰ The meta-analysis showed geographic variability in HPV prevalence. Among Asian counties, China and South Korea showed the highest HPV prevalence (13.6%) compared to Taiwan and Japan (7.0%); the Philippines, Thailand and Vietnam (7.2%); and India (7.5%). The authors inferred that this high HPV prevalence in eastern Asia might be related to liberal sexual behavior patterns.

Prior reports conducted in different regions of South Korea have shown variable HPV infection rates in women with normal cytology: 8.5% (75/751) in Busan, in a study using PCR-EIA (enzyme immunoassay) to detect 36 HPV types,¹³ 10% (13/130) in Incheon, in a study using PCR with consensus primers to detect seven HR-HPV types,¹⁴ and 19.4% (84/432) in Bucheon, Gyeonggi-do¹⁵ or 35.1% (401/1143) in Bundang, Gyeonggi-do,¹⁶ in studies using HPV DNA chip testing (Biomedlab, Seoul, Korea) to detect 22 HPV types. Among these studies, two were performed in similar areas, but in different locations-Bucheon, Gyeonggi-do and Bundang, Gyeonggi-do- using the same HPV DNA chip kit (Biomedlab). These studies revealed different HPV-infection rates (19.4% vs 35.1%).^{15,16} In our study, the overall HPV-positivity rates were similar (15.1-17.8%) across the five regions. However, widely varying HPV infection rates was evident in the hospital studied (9.7- 22.8%), even though all of the participating institutions were university or referral hospitals. HR-HPV prevalence was also variable across the regions. The variability of HPV-positivity rates by hospital and region may be partly related to differences in the nature of

the patients as well as patient age and sexual activity between hospitals and regions.

HPV prevalence in women with abnormal cytology

This study demonstrated that overall HPV prevalence is much higher in women with abnormal cytology (78.4%) than in women with normal cytology (15.8%) ($p=0.00$). The HPV prevalence in SIL+/AIS+ women (LSIL and HSIL+/AIS+: 91.3%, 211 of 231) was significantly higher than that in women with ASC/AGC (ASC-US and ASC-H/AGC: 61.6%, 109 of 177) ($p=0.00$). There was no significant difference, however, between women with ASC-US (60.4%) and ASC-H/AGC (67.3%), and between women with LSIL (90.7%) and HSIL+/AIS+ (91.7%) ($p>0.05$). In contrast, the prevalence of HR-HPV was different by cytological interpretation, showing a higher correlation with cytological atypia. As might be expected, the HR-HPV frequency in SIL+/AIS+ women (LSIL and HSIL+/AIS+: 66.2%, 153 of 231) was significantly higher than that detected in women with ASC/AGC (ASC-US and ASC-H/AGC: 36.2%, 64 of 177) ($p=0.00$). Also, the HR-HPV infection rate was significantly higher in women with HSIL+/AIS+ (73.8%) compared to women with LSIL (53.5%) ($p=0.00$). Although the difference in prevalence between women with ASC-US (34.2%) and ASC-H/AGC (46.4%) was not statistically significant ($p>0.05$), HR-HPV prevalence in women with ASC-H/AGC (46.4%) was intermediate between that of women with ASC-US (34.2%) and HSIL+/AIS+ (73.8%). These results are similar to those of other studies.¹⁷⁻¹⁹ ASC-H cytology, defined as cytological changes suggestive of HSIL, would likely be associated with a higher positive predictive value for the detection of underlying CIN2 or 3 than ASC-US, but would be less predictive than HSIL. In women with ASC-H, frequencies of HR-HPV DNA detection and underlying high-grade CIN have been reported to be intermediate between those of ASC-US and HSIL.¹⁷ On the other hand, CIN2 or 3 has been identified significantly more frequently in HR-HPV-positive ASC-H (32.7%) than in HR-HPV-negative ASC-H (1.2%).¹⁸ HR-HPV can be associated with both LSIL and HSIL, but it should be observed with significantly higher frequency in women with HSIL.^{3,19} These data suggest that HR-HPV types are closely related to the development of high-grade CIN and IC of the uterine cervix.

In our study, the overall prevalence of HR-HPV infection in women with LSIL was 53.5%, with a wide range of HR-HPV positivity rates across regions (40.4-87.5%). Our HR-HPV infection rate result was lower than the ALTS result (83%).²⁰ More-

over, the ALTS group described a much narrower range of HR-HPV-DNA positivity among women with LSIL (79.1-86.1%) than the range reported in our study; this may be related to the ages of the participants. The ALTS study population was young (mean age, 24.9-years-of-age) and only 17.6% of women with LSIL in their study were ≥ 30 -years-of-age. In our study, however, the population of women with LSIL (mean age, 42-years-of-age) had a wide age range, with only 16.3% (14 of 86) <30 years old and 58.1% (50 of 86), the majority, ≥ 40 -years-of-age. Compared with younger women, older women are less likely to be HR-HPV positive and more likely to have false-positive LSIL cytology.^{21,22} While a cytological interpretation of LSIL could be a very accurate marker for HPV infection in young women, many cases of LSIL in postmenopausal women could represent epithelial alterations of atrophic changes. The peri- or post-menopausal uterine cervix exhibits pseudokoilocytosis, including prominent perinuclear halos, nuclear hyperchromasia, variation in nuclear size and multinucleation, while showing low positivity for HPV DNA.²¹ In older women with LSIL, the low HR-HPV detection rate is thought to be associated with age-related epithelial alterations, termed postmenopausal squamous atypia.²² Therefore, our data demonstrating a relatively low prevalence and wide range of HR-HPV positivity in women with LSIL cytology could be explained by age-related phenomena. Based on this evidence, well-standardized HPV DNA testing on age-adjusted LSIL or HSIL samples could potentially be utilized as an alternative method for quality-control purposes in cytology laboratories.

In regard to regional variability in women with ASC/AGC positive for HR-HPV, our data also showed a wide range of HR-HPV positivity in women with ASC-US (25.0-60.0%) and ASC-H/AGC (35.7-80.0%). Similarly, the ALTS group showed a wide range of HR-HPV positivity among women with ASC-US (31-60%).²³ Regional variability in the ASC category is largely caused by the cytological characteristics of ASC classification, because ASC encompasses heterogeneous biologic entities, from normal cytology unrelated to HR-HPV infection to, rarely, IC that is strongly related to HR-HPV infection.

HPV type-specific prevalence

In this study, HPV type-specific prevalence between women with normal and abnormal cytology was significantly different ($p=0.00$). The five most common HPV types in women with abnormal cytology were HPV16, 58, 18, 52, and 56 or 53. Among those, the prevalence of HPV16 (20.6%) was much higher than that of HPV58 (10.8%), HPV18 (8.0%) and HPV52

(4.9%). HPV16 was the most common viral type found in most abnormal cytological categories, including LSIL, HSIL/AIS, and IC. Increased HPV16 prevalence was correlated with an increased severity of cytological atypia: women with normal cytology, 6.8%; ASC/AGC, 11.5%; LSIL, 12.4%; HSIL/AIS, 31.4%; and IC, 37.2% ($p=0.00$). These observations suggest that HPV16 is the most common type of the HR-HPV and also the single most commonly identified HPV type in high-grade intraepithelial lesions and cancers.

Overall, the five most common types of HPV presently detected were 16, 58, 18, 33, and 52 in women with HSIL/AIS, compared to HPV16, 18, 31 or 58, and 45 or 52 in IC. Among these HPV types, HPV16, 18, 31, and 45 were more prevalent in IC than HSIL/AIS. These results are consistent with a previous meta-analysis, in which HPV16, 18, and 45 were each more prevalent in women with invasive SCC than in women with HSIL, suggesting that an HSIL infected with HPV16, 18, or 45 preferentially progresses to SCC.²⁴ Our data also suggest that HPV16-, 18-, 31-, or 45-infected HSIL preferentially progresses to SCC, although our number of study cases was too small for proper evaluation.

A worldwide meta-analysis reported geographic differences in HPV type-specific prevalence.¹¹ In SCC, HPV16, 18, 45, 31, and 33 were the five most common HPV types in all regions except Asia. In Asia, HPV types 58 and 52 were more frequently identified, especially in eastern and southeastern areas. Other studies in Asia and Korea have presented the same data.^{25,26} Our study data confirms these previous reports. HPV types 58 and 52 were frequently identified as one of the five most common HPV types in women with normal and abnormal cytology. HPV types 16, 18, 52, and 58 occupied 58.5% of all HPV infections in women with HSIL/AIS, and 65.1% of all HPV infections in women with IC. Based on this data, HPV types 58 and 52 should also be considered in the development of HPV vaccines for Korean women.

Type-specific prevalence in women with normal cytology also has significant geographic regional variations.^{10,27} In normal cytology specimen the proportion of the most common HPV16 was variable across regions (12.3-25.5%), and heterogeneity between areas of Asia was significant.²⁷ Similarly, our results showed heterogeneity of HPV types across regions in women with normal cytology ($p>0.05$). The five most common types were HPV18, 53, 70, 16, and 52 or 58 in normal cytology, but the rank of HPV prevalence by type was variable across the regions. In contrast, abnormal cytological specimens showed homogeneity in HPV-type distribution across the regions. HPV16 was significantly the most common type in all regions except one (Busan) ($p=$

0.00). Our results support the findings of another study, which demonstrated that regional heterogeneity decreases with increasing lesion severity as HPV16 becomes increasingly dominant.²⁸

In summary, a total of 2,368 participants were gathered from five regions. The mean participant age was 45.7 years. The HPV chip test provided useful data regarding HPV positivity and viral type. Overall HPV and HR-HPV positivity were 15.8% and 8.0% in women with normal cytology, 78.4% and 53.2% in women with abnormal cytology, and 91.7% and 73.8% in women with HSIL+/AIS+. HR-HPV positivity showed better correlation with high-grade cytological atypia than did overall HPV positivity, which included non-HR-HPV types. The prevalence of overall HPV in cases of LSIL (90.7%) was similar to that found in cases of HSIL+/AIS+ (91.7%), but HR-HPV prevalence was much higher in women with HSIL+/AIS+ (73.8%) than in women with LSIL (53.5%) ($p=0.00$). This data suggest that HR-HPV types are closely related to the development of the cervical cancer. In descending order of frequency, the five most common HPV types in women with normal cytology were 18, 53, 70, 16, and 52 or 58, and HPV16, 58, 18, 52, and 56 or 53 in women with abnormal cytology. HPV type-specific distribution was correlated with cytological diagnosis. HPV16 was the most common type in abnormal cytological categories, including LSIL, HSIL/AIS, and IC across most regions. However, women with normal cytology showed increased heterogeneity of HPV type distribution than those with abnormal cytology ($p>0.05$). Moreover, contrary to the findings in western countries, HPV58 and 52 were frequently identified as one of the five most common HPV types in women with both normal and abnormal cytology. HPV16, 18, 31, and 45 were more prevalent in IC than in HSIL/AIS, which supports findings that HSIL/AIS infected with HPV16, 18, 31, or 45 preferentially progresses to SCC. Although our study number of abnormal cytology specimens across the regions was not large enough for proper evaluation, our overall findings are consistent with other studies previously cited. Our results may provide important information in the development of cervical screening and vaccination programs.

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