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Original Article

Prevalence of Human Papillomavirus Genotypes in Routine Pap Smear of 2,562 Korean Women Determined by PCR-DNA Sequencing

Kyung-Ok Lee*, Su-Jin Jeong, Min-Young Park, Hye-Soon Seong, Eun-Sim Shin, Kyeong-Hwan Choi, Gee-Young Kim and Sun-Hwa Lee

Genome Research Center, Neodin Medical Institute, Seoul, Korea

The infections by human papillomaviruses (HPVs) are clearly associated with the subsequent development of cervical cancer. In this study, HPV genotype distribution and prevalence were detected in Korean women from January to December 2008 using PCR-DNA sequencing. A total of 2,562 cervical samples from Korean women having routine Pap smear cytology screening were used. HPV DNA was extracted from cervical swab samples and amplified by PCR in L1 region of HPV. HPV DNA was detected in 23.2% and 65.5% from the groups of normal and abnormal Pap cytology, respectively. The prevalence of high-risk types of HPV had the highest frequency in the <30 year-olds' group (50.6%). The prevalence of HPV in normal, ASCUS, LSIL and HSIL groups was 23.2%, 58.1%, 96.3% and 97.0%, respectively. Moreover, the frequencies of the high-risk types of HPV were 16.2% in the normal Pap cytology, 44.7% in the ASCUS, 76.1% in the LSIL and 94.1% in the HSIL groups. The prevalence of the high-risk types of HPV increased in proportion to the severity of the cytological classification. In the HSIL group, HPV type 16 was the most frequently found at 32.4%, followed by types 58, 53 and 33 at 17.6%, 14.7% and 11.8%, respectively. HPV type 82 was found in 5.6% of the HSIL group and was not detected in the normal Pap cytology group. The frequency of high-risk type of HPV 82 is firstly reported in Korean women. This finding could be an informative basis for the development of future HPV vaccination strategies in Korean women.

Key Words: HPV genotype, Prevalence, PCR-DNA sequencing

INTRODUCTION

Cervical cancer is one of the most common malignancies and is the major cause of cancer mortality. Clinical and epidemiological studies have shown that the human papillomaviruses (HPVs) are the major infectious etiologic agents of genital precancerous lesions and cancers (1). HPVs are strictly epitheliotropic viruses infecting cutaneous or mucosal surfaces and display a very high selectivity for the specific epithelium infected (2). The persistence of an HPV infection favors viral integration in the cell genome, which, together with other factors, can progress to high-grade, squamous intra-epithelial lesions (HSIL) and cancers (3).

More than 100 different HPV types have been described, of which at least 30 have been identified in the female genital tract and associated with epithelial neoplasm, ranging from benign common warts to malignant carcinoma of the uterine cervix (4). HPVs are classified into low- and high-risk categories, based on their association with malignant lesions and phylogenetic relationships (5). Currently, 19 HPV types, including 16, 18, 26, 30, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73 and 82 are classified as high-risk types (6). High-risk types have been shown to cause more than 99% of all cases of cervical cancer. Identification

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*Corresponding author: Kyung Ok Lee, Ph.D., Genome Research Center, Neodin Medical Institute, #2-3, Yongdap-Dong, Sungdong-gu, Seoul, 133-847, Korea.
Phone: +82-2-2244-6500, Fax: +82-2-2212-1307,
e-mail: grace97284@yahoo.co.kr

of high-risk HPV genotypes may permit selecting those patients who are at an increased risk for disease and may provide additional clinical value (7).

Knowledge of HPV status is becoming increasingly important as a triage screen after the detection of atypical cells of undetermined significance and as a primary screen for cervical cancer detection. Adequate detection and genotyping are needed to diagnose and study the role of HPV infections in individual patients and patient populations (8).

Usually, a Pap smear has been used as a cervical cancer screening test. However, the sensitivity of the Pap smear does not exceed 50% (9). Until now, there has not been a serological test to detect the presence of HPV in cervical specimens (10). However, sensitive and specific methods are available, based on the detection of HPV DNA, a variety of hybridization techniques, dot blots (11, 12), reverse hybridization line probe assays (13, 14), DNA microarray (15~18) and PCR-direct sequencing (19, 20). A general drawback of hybridization techniques is that they may result in cross-hybridizations of closely related genotypes as well as non-specific hybridization (21, 22). The DNA sequencing method for HPV genotyping can be applied easily to the analysis of tissue samples and it allows type-specific follow-ups for women who have been treated for cervical intraepithelial neoplasia. To apply the DNA sequencing method in routine clinical laboratory, an expensive machine is required and it will take 2~3 days from request to report (19). The prevalence of different HPV genotypes depends on the severity of disease, the target population and the geographic area (24). The aim of this study was to assess the type-specific prevalence of HPV using a sequence-based typing in a Korean population. The association of HPV genotypes with the cytological results was also evaluated.

MATERIALS AND METHODS

Study populations

After a routine Pap smear for cytological evaluation, an additional cervical swab sample was taken in 2,562 Korean women from January to December 2008. The cervical samples used in this study were collected anonymously from Korean women who visited regional hospitals for a routine Pap smear screening. Among these, 45 samples were not informed about age. The average age of sample was 37.6 year-old (range: 20~73 year-old). Samples were classified with four age groups: <30 year-olds' group, 30~40 year-olds' group, 40~50 year-olds' group and >50 year-olds' group.

Cytology

All Pap smears were analyzed in cytological laboratories at the Neodin Medical Institute, Korea. Cytology was classified according to the 2001 Bethesda System into five categories: negative, atypical squamous cells undetermined significance (ASCUS), low-grade squamous intraepithelial lesion (LSIL), high-grade squamous intraepithelial lesion (HSIL) and invasive cancer (23).

HPV PCR

Cervical swab samples were centrifuged at 2,000 g and supernatant was removed. DNA was extracted from 200 μl of concentrated sample using a DNA purification kit (Intron Co., Sungnam, Korea). Viral gene-spin lysis buffer (250 μl) was added to sample and incubated at room temperature for 10 min and 350 μl of binding buffer was added. Lysate was loaded on a spin column and centrifuged at 13,000 rpm for 1 min. After discarding the solution, 500 μl of washing buffer A was added to the column and centrifuged for 1 min at 13,000 rpm. After discarding the solution, 500 μl of washing buffer B was added to the column and centrifuged for 1 min at 13,000 rpm. After discarding the solution, the spin column was placed in a RNase-free 1.5 ml micro-centrifuge tube, and elution buffer (30 μl) was directly added onto the membrane and incubated at room temperature for 1 min. Eluted solution (2~5 μl) was used for PCR. For amplification of HPV DNA, PCR primers were selected in the HPV L1 gene (25). Primer sequences are 5'-TTTG-TTACTGTGGTAGATAC-3 and 3'-ACTAAATGTCAAA-TAAAAAG-5'. The PCR reaction mixture contained 4.5 μl of DNA template, 0.05 μl of PCR primers, 1 μl of dNTPs (2.5 mM/L), 0.2 μl of *Taq* polymerase (5 U/μl), 4 μl of 10 × buffer and 10.2 μl of distilled water in a total volume of

Table 1. Age distribution and prevalence of high- or low-risk HPVs in Koreans

	>30 years	31~40 years	41~50 years	>50 years
High-risk types HPV[a]	37.8% (220/582)	31.3% (271/867)	29.8% (207/699)	23.0% (85/369)
Low-risk types HPV[b]	10.3% (60/582)	5.4% (47/867)	5.6% (39/699)	7.9% (29/369)
Uncharacterized	2.6% (15/582)	2.4% (21/867)	2.0% (14/699)	3.0% (11/369)
Total % of HPV	50.6% (295/582)	39.1% (339/867)	37.4% (261/699)	34.7% (125/369)

[a] High-risk types of HPV: 16, 18, 26, 30,31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, 82
[b] Low-risk types of HPV : 6, 11, 22, 32, 34, 40, 42, 43, 44, 54, 61, 62, 72, 81

20 μl. The amplification conditions included an initial denaturation for 3 min at 95℃, 35 cycles of amplification with denaturation at 94℃ for 45 sec, annealing at 55℃ for 45 sec, extension at 72℃ for 45 sec, followed by final extension at 72℃ for 5 min. PCR products were detected by 2% agarose gel electrophoresis.

Sequencing of PCR products

Amplified PCR products were purified on Wizard PCR Preps DNA purification resin (Promega, Madison, WI, USA), and sequenced bidirectionally with Dye Terminator Cycle Sequencing Ready Reaction kit (PE Applied Biosystems, Foster City, CA, USA). Sequencing was performed on an automated DNA sequencer ABI 3730 (PE Applied Biosystems). Obtained sequences of HPV isolates from the patients were compared with documented virus sequences available in GenBank by using the BLAST program (26). The usual Chi-square test was used to analyze the significance of the different prevalence of HPV genotypes in cervical samples from normal control group and HSIL group. For all analyses, $p < 0.05$ was considered statistically significant.

RESULTS

Prevalence of HPV among age groups

The prevalence of HPV was 50.6%, 39.1%, 37.4% and 34.7% in the <30 year-olds' group, 31~40 year-olds' group, 41~50 year-olds' group and >50 year-olds' group, respectively (Table 1). The prevalence of carcinogenic HPV types and total HPV were the highest in <30 year-olds' group and gradually low in older age groups. Low-risk type HPVs were also frequently found in <30 year-olds' group (10.3%) (Table 1).

Prevalence of HPV in normal and abnormal cytology groups

In the present study, normal and abnormal cytological results with a Pap smear were found in 59.7% and 40.3%, respectively. Invasive cancer sample in cytological examination was not found. As expected, this study shows that women infected with carcinogenic HPV genotypes are at significantly increased risk of having abnormal cytology. HPV type 16 was the most commonly found genotype in women in both the normal cytology group (3.9%) and the abnormal cytology group (13.8%). Overall HPV prevalence among the 2,562 women included in this analysis was 40.2% (1,031/2,562). Positive rates of HPV were 23.2% and 65.5% in the normal cytology group and the abnormal cytology group, respectively. The high-risk type of HPV was more frequent in the abnormal cytology group (51.3%) than in the normal cytology group (16.2%) (Table 2).

Genotype-specific HPV prevalence in cytologically classified groups

The prevalence of HPV in the normal, ASCUS, LSIL and HSIL groups was 23.2%, 58.1%, 96.3% and 97.0%, respectively. In the present study, 30 HPV types were found, including 17 high-risk types and 13 low-risk types. Among 19 high-risk HPV types reported until recently, HPV types 45 and 73 were not found in Koreans. The prevalence of high-risk type HPV versus the low-risk type HPV was as follows: 16.2% vs. 5.6% in the normal cytology group, 44.7% vs. 9.0% in the ASCUS group, 76.1% vs. 15.3% in

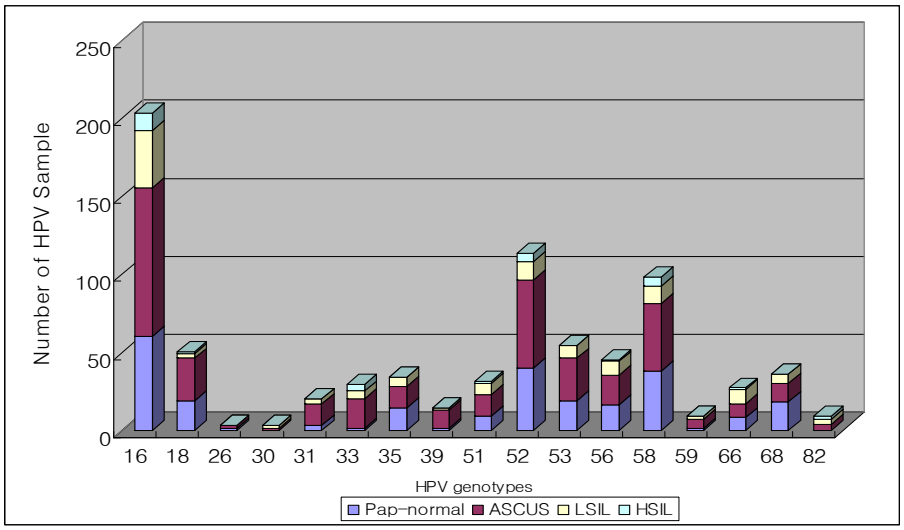
Table 2. Type-specific HPV prevalence determined by PCR-sequencing in normal and abnormal cytology groups

	Pap-normal		Pap-abnormal		ASCUS		LSIL		HSIL		Total		*P* value
	n	%	n	%	n	%	n	%	N	%	n	%	
Total No	1529	59.7	1033	40.3	836	32.6	163	6.4	34	1.3	2562	100	
Any type	355	23.2	676	65.5	486	58.1	157	96.3	33	97.0	1031	40.2	<0.05
High risk types of HPV (Carcinogenic types)													
16[a]	60	3.9	143	13.8	95	11.4	37	22.7	11	32.4	203	7.9	<0.05
18	19	1.2	31	3.0	27	3.2	3	1.8	1	2.9	50	2.0	[c]NS
26	1	0.1	2	0.2	2	0.2	0	0.0	0	0.0	3	0.1	NS
30	0	0.0	3	0.3	1	0.1	2	1.2	0	0.0	3	0.1	NS
31	3	0.2	17	1.6	14	1.7	3	1.8	0	0.0	20	0.8	NS
33[a]	1	0.1	28	2.7	19	2.3	5	3.1	4	11.8	29	1.1	<0.05
35	14	0.9	20	1.9	14	1.7	6	3.7	0	0.0	34	1.3	NS
39	1	0.1	13	1.3	12	1.4	1	0.6	0	0.0	14	0.5	NS
51	9	0.6	22	2.1	14	1.7	7	4.3	1	2.9	31	1.2	NS
52[a]	40	2.6	73	7.1	56	6.7	12	7.4	5	14.7	113	4.4	<0.05
53	19	1.2	35	3.4	27	3.2	8	4.9	0	0.0	54	2.1	NS
56	16	1.0	29	2.8	19	2.3	9	5.5	1	2.9	45	1.8	NS
58[a]	38	2.5	60	5.8	43	5.1	11	6.7	6	17.6	98	3.8	<0.05
59	1	0.1	8	0.8	6	0.7	2	1.2	0	0.0	9	0.4	NS
66	8	0.5	19	1.8	9	1.1	9	5.5	1	2.9	27	1.1	NS
68	18	1.2	18	1.7	12	1.4	6	3.7	0	0.0	36	1.4	NS
82[a]	0	0.0	9	0.9	4	0.5	3	1.8	2	5.9	9	0.4	<0.05
subtotal	248	16.2	530	51.3	374	44.7	124	76.1	32	94.1	778	30.4	[b]<0.0001
Low risk types of HPV													
6	10	0.7	7	0.7	2	0.2	5	3.1	0	0.0	17	0.7	NS
11	1	0.1	4	0.4	1	0.1	3	1.8	0	0.0	5	0.2	NS
32	2	0.1	1	0.1	1	0.1	0	0.0	0	0.0	3	0.1	NS
34	2	0.1	4	0.4	4	0.5	0	0.0	0	0.0	6	0.2	NS
40	2	0.1	2	0.2	2	0.2	0	0.0	0	0.0	4	0.2	NS
42	0	0.0	4	0.4	2	0.2	2	1.2	0	0.0	4	0.2	NS
43	0	0.0	9	0.9	8	1.0	1	0.6	0	0.0	9	0.4	NS
54	14	0.9	7	0.7	6	0.7	0	0.0	1	2.9	21	0.8	NS
61	12	0.8	16	1.5	12	1.4	4	2.5	0	0.0	28	1.1	NS
62	16	1.0	17	1.6	13	1.6	4	2.5	0	0.0	33	1.3	NS
70	21	1.4	19	1.8	15	1.8	4	2.5	0	0.0	40	1.6	NS

Table 2. Continued

Low risk types of HPV												
81	3	0.2	8	0.8	6	0.7	2	1.2	0	0.0	11	0.4	NS
84	2	0.1	3	0.3	3	0.4	0	0.0	0	0.0	5	0.2	NS
subtotal	85	5.6	101	9.8	75	9.0	25	15.3	1	2.9	186	7.3	NS
Uncharacterized	22	1.4	45	4.4	37	4.4	8	4.9	0	0.0	67	2.6	

Note: ASCUS; atypical squamous cells undetermined significance, LSIL; low-grade squamous intraepithelial lesion, HSIL; high-grade squamous intraepithelial lesion.
[a]: P value was observed between normal cytology group and HSIL group. Five high-risk types of HPV (HPV-16, 33, 52, 58, 82) were statistically significant between two groups.
[b]: Positive rate of high risk type of HPV of HSIL is statistically significant than normal cytology group ($p < 0.0001$).
[c]: NS: non significant



* ASCUS: atypical squamous cells undetermined significance, LSIL: low-grade squamous intraepithelial lesion, HSIL: high-grade squamous intraepithelial lesion.

Figure 1. Distribution of carcinogenic HPV types in cytological normal women and abnormal women, including ASCUS, LSIL and HSIL, respectively.

the LSIL group and 94.1% vs. 2.9% in the HSIL group. The frequency of high-risk types of HPV increased in a proportion to the severity of cytological classification, and it was the most prominent in the HSIL group (Table 2). Fig. 1 shows the distribution of high-risk HPV types according to cytology groups. Prevalence of high-risk HPV between normal group and HSIL group was statistically significant ($p < 0.0001$). When we compared the normal cytology group and HSIL group, five high-risk HPV types, including types 16, 33, 53, 58 and 82, were statistically significant ($p < 0.05$). In the HSIL group, type 16 was the most frequently found at a frequency of 32.4%, followed by types 58, 53, 33 and 82 at 17.6%, 14.7%, 11.8% and 5.9%, respectively. These five HPV types accounted for 82.0% of the high-risk HPV in HSIL and they were followed by four HPV types, including types 18, 51, 56 and 66. In particular, the HPV type 82 was relatively frequently found in the HSIL group, but it was not found at all in the normal cytology group ($p < 0.05$). Low-risk types of HPV were 5.6 % in the normal cytology group and 9.8% in the abnormal cytology group. Among abnormal cytology groups, low-risk types of HPV were the most frequently found in LSIL (15.3%) group, but it was found only 2.9% in HSIL group (Table 2).

DISCUSSION

HPVs are etiological agents for cervical cancer. The investigation of HPV infection is clinically important for the management of cervical cancers (8). In the investigation of HPV infections in various age groups of Korean women, the prevalence of total HPV and high-risk types of HPV were highest in the <30 year-olds' group and decreased in the >50 year-olds' group (Table 1). This study showed a continuous decline in the prevalence rates of infections with increasing age. This result was similar as the previous reports from Italy (27) and Germany (9). The prevalence of HPV increases strongly in the worst-case cytological diagnosis, from 23.2% in the cytological normal group up to 97.0% in the worst Pap classification. The distribution of carcinogenic HPV types in Korean women showed marked differences between the cytologically normal group (16.2%) and the HSIL (94.1%). In particular, the prevalence of HPV type 16 (32.4%) in the HSIL was significantly higher than in the normal cytology group (3.9%) ($p < 0.05$) (Table 2). In the present study, carcinogenic HPV was the most frequent in the HSIL group (94.1%), and the prevalence of low-risk types of HPV was the most frequent in the LSIL group (15.3%), and it corresponded to the reports in German people (9). Some geographical variations in the distribution HPV types have been reported, with frequencies ranging from 3% to 30% or more (6). In this study, prevalence of HPV was 40.2% of the cytology screened 2,562 women and it was similar to the previous data in 140 Korean samples (44.8%) (29).

Kim et al. (28) reported that three high-risk types of HPVs, including HPV 16 (42.9%), 58 (18.4%) and 31 (14.3%), comprised the majority (75.5%) of the HPV found in cervical cancer samples in Korean women. Moreover Kahng et al. (30) reported that HPV types 16, 58, 31, 18, 35, 33 and 52 were found in descending order of frequency in 212 cervical cancer samples by using microarray method in Koreans. In the present study, types 16 (32.4%), 58 (17.6%), 52 (14.7%), 33 (11.8%) and 82 (5.9%) were frequently detected in the HSIL group; however, HPV types 31 and 35 were not found (Fig. 1). HPV type 82 was not found in normal cytology group, but it was found in 0.5% of the ASCUS group, 1.8% of the LSIL group and 5.9% of the HSIL group, respectively. The relationship between HPV type 82 and cancer has not been reported in any of the previous Korean studies (15, 16, 18, 23, 28~30). Carcinogenic HPV type 82 in Korean women could be found by using a sequencing method that could improve the genotyping of updated HPV types recently found and knowledge of the epidemiological distribution of HPV types. Detection of HPV type 82 is not available by using microarray kits which were not included type 82 specific probe (Biocore, Seoul, Korea; Biomedlab, Seoul, Korea).

The prevalence of HPV type could be different in ethnic groups. HPV types observed with the worst-case cytological diagnoses in Germany were types 16 (55.6%), 18 (33.3%), 35 (22.2%), 39 (11.1%), 52 (11.1%), and 58 (11.1%) (18). In the present study, HPV types 16, 52 and 58 were also found in Korean women. However, HPV types 35 and 39 were not detected in the HSIL group. Moreover, HPV type 18, which is generally considered to be related to calcinogenesis in a previous report (9), was only found in 2.9% of the HSIL group. It seems that HPV 18 does not have a major role in carcinogenesis of cervical cancer in Korean women.

A mixed infection could be another issue for detecting HPV genotypes. The prevalence of mixed HPV infections by DNA microarray was reported in 20% of the 2,470 Korean women (23). In the present study, we separated the mixed infections into another group, namely, the uncharacterized group, which accounted for 7.3% of the total women (186/2,562) (Table 2). The persistence of HPV infections bears a high risk for the recurrence of cancer, but only type-specific analysis can differentiate between the true persistence of a specific type or a new HPV infection (20). Clear identification of HPV genotypes could have important prognostic or therapeutic value, as it can distinguish between HPV types of high and low oncogenic risks. Updated information on HPV type-specific prevalence in Korean women is necessary to assess the prevalence of HPV genotypes targeted by available vaccines and to

estimate the potential impact of the vaccinations.

REFERENCES

1) Bosch FX, Manos MM, Munoz N, Sherman M, Jansen AM, Peto J, Schiffman MH, Moreno V, Kurman R, Shah KV. Prevalence of human papillomavirus in cervical cancer: a worldwide perspective. International biological study on cervical cancer (IBSCC) Study Group. J Natl Cancer Inst 1995;87:796-802.
2) zur Hausen H. Papillomaviruses causing cancer: evasion from host-cell control in early events in carcinogenesis. J Natl Cancer Inst 2000;92:690-8.
3) Lazo PA. Papillomavirus integration: prognostic marker in cervical cancer? Am J Obstet Gynecol 1997;176: 1121-2.
4) McGlennen RC. Human papillomavirus oncogenesis. Clin Lab Med 2000;20:383-406.
5) Lorincz AT, Reid R, Jenson AB, Greenberg MD, Lancaster W, Kurman RJ. Human papillomavirus infection of the cervix: relative risk associations of 15 common anogenital types. Obstet Gynecol 1992;79: 328-37.
6) Munoz N, Bosch FX, de Sanjose S, Herrero R, Castellsague X, Shah KV, Snijders PJ, Meijer CJ. Epidemiologic classification of human papillomavirus types associated with cervical cancer. N Engl J Med 2003;348:518-27.
7) Walboomers JM, Jacobs MV, Manos MM, Bosch FX, Kummer JA, Shah KV, Snijders PJ, Peto J, Meijer CJ, Munoz N. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. J Pathol 1999; 189:12-9.
8) Bollmann R, Mehes G, Torka R, Speich N, Schmitt C, Bollmann M. Determination of features indicating progression in atypical squamous cells with undetermined significance: human papillomavirus typing and DNA ploidy analysis from liquid-based cytologic samples. Cancer 2003;99:113-7.
9) Klug SJ, Hukelmann M, Hollwitz B, Duzenli N, Schopp B, Petry KU, Iftner T. Prevalence of human papillomavirus types in women screened by cytology in Germany. J Med Virol 2007;79:616-25.
10) Weintraub J. Experience with new technologies within the context of Swiss practice and conclusions. Ann Pathol 1999;19:S96-8.
11) Jacobs MV, de Roda Husman AM, van den Brule AJ, Snijders PJ, Meijer CJ, Walboomers JM. Group-specific differentiation between high- and low-risk human papillomavirus genotypes by general primer-mediated PCR and two cocktails of oligonucleotide probes. J Clin Microbiol 1995;33:901-5.
12) Ylitalo N, Bergstrom T, Gyllensten U. Detection of genital human papillomavirus by single-tube nested PCR and type-specific oligonucleotide hybridization. J Clin Microbiol 1995;33:1822-8.
13) Coutlee F, Gravitt P, Kornegay J, Hankins C, Richardson H, Lapointe N, Voyer H, Franco E. Use of PGMY primers in L1 consensus PCR improves detection of human papillomavirus DNA in genital samples. J Clin Microbiol 2002;40:902-7.
14) van den Brule AJ, Pol R, Fransen-Daalmeijer N, Schouls LM, Meijer CJ, Snijders PJ. GP5+/6+ PCR followed by reverse line blot analysis enables rapid and high-throughput identification of human papillomavirus genotypes. J Clin Microbiol 2002;40:779-87.
15) Choi BS, Kim O, Park MS, Kim KS, Jeong JK, Lee JS. Genital human papillomavirus genotyping by HPV oligonucleotide microarray in Korean commercial sex workers. J Med Virol 2003;71:440-5.
16) Choi YD, Jung WW, Nam JH, Choi HS, Park CS. Detection of HPV genotypes in cervical lesions by the HPV DNA Chip and sequencing. Gynecol Oncol 2005; 98: 369-75.
17) Klaassen CH, Prinsen CF, de Valk HA, Horrevorts AM, Jeunink MA, Thunnissen FB. DNA microarray format for detection and subtyping of human papillomavirus. J Clin Microbiol 2004;42:2152-60.
18) Oh TJ, Kim CJ, Woo SK, Kim TS, Jeong DJ, Kim MS, Lee S, Cho HS, An S. Development and clinical evaluation of a highly sensitive DNA microarray for detection and genotyping of human papillomaviruses. J Clin Microbiol 2004;42: 3272-80.
19) Lee KO, Seong HS, Chung SJ, Jung NY, Lee HJ, Kim KT. Genotype frequency of human papillomavirus determined by PCR and DNA sequencing in Korean women. Korean J Clinical Lab Sci 2006;38:99-104.

20) Speich N, Schmitt C, Bollmann R, Bollmann M. Human papillomavirus (HPV) study of 2916 cytological samples by PCR and DNA sequencing: genotype spectrum of patients from the west German area. J Med Microbiol 2004;53:125-8.
21) Cope JU, Hildesheim A, Schiffman MH, Manos MM, Lorincz AT, Burk RD, Glass AG, Greer C, Buckland J, Helgesen K, Scott DR, Sherman ME, Kurman RJ, Liaw KL. Comparison of the hybrid capture tube test and PCR for detection of human papillomavirus DNA in cervical specimens. J Clin Microbiol 1997;35:2262-5.
22) Coste-Burel M, Besse B, Moreau A, Imbert BM, Mensier A, Sagot P, Lopes P, Billaudel S. Detection of human papillomavirus in squamous intraepithelial lesions by consensus and type-specific polymerase chain reaction. Eur J Obstet Gynecol Reprod Biol 1993; 52:193-200.
23) Hwang HS, Park M, Lee SY, Kwon KH, Pang MG. Distribution and prevalence of human papillomavirus genotypes in routine Pap smear of 2,470 Korean women determined by DNA Chip. Cancer Epidemiol Biomarkers Prev 2004;13:2153-6.
24) Cuzick J. Human papillomavirus testing for primary cervical cancer screening. JAMA 2000;283:108-9.
25) de Roda Husman AM, Walboomers JM, Van den Brule AJ, Meijer CJ, Snijders PJ. The use of general primers GP5 and GP6 elongated at their 3' ends with adjacent highly conserved sequences improves human papillomavirus detection by PCR. J Gen Virol 1995;76:1057-62.
26) Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 1997;25:3389-402.
27) Agarossi A, Ferrazzi E, Parazzini F, Perno CF, Ghisoni L. Prevalance and type distribution of high-risk human papillomavirus infection in women undergoing voluntary cervical cancer screening in Italy. J Med Virol 2009;81:529-35.
28) Kim KH, Yoon MS, Na YJ, Park CS, Oh MR, Moon WC. Development and evaluation of a highly sensitive human papillomavirus genotyping DNA chip. Gynecol Oncol 2006;100:38-43.
29) Kim CJ, Jeong JK, Park M, Park TS, Park TC, Namkoong SE, Park JS. HPV oligonucleotide microarray-based detection of HPV genotypes in cervical neoplastic lesions. Gynecol Oncol 2003;89: 210-7.
30) Kahng J, Lee HJ. Clinical efficacy of HPV DNA chip test in the era of HPV vaccination. Korean J Lab Med 2008;28:70-8.