# Correlation of Cervical Carcinoma and Precancerous Lesions with Human Papillomavirus (HPV) Genotypes Detected with the HPV DNA Chip Microarray Method

**Hee Jung An, M.D.**[1]
**Nam Hoon Cho, M.D.**[2]
**Sun Young Lee, M.D.**[3]
**In Ho Kim, M.D.**[3]
**Chan Lee, M.D.**[3]
**Seung Jo Kim, M.D.**[3]
**Mi Sook Mun, C.T. (I.A.C.)**[1]
**Se Hyun Kim, Ph.D.**[4]
**Jeongmi Kim Jeong, Ph.D.**[5]

[1] Department of Pathology, College of Medicine, Pochon CHA University, Kyonggi-do, South Korea.

[2] Department of Pathology, Yonsei University, Seoul, South Korea.

[3] Department of Gynecologic Oncology, Pochon CHA University, Kyonggi-do, South Korea.

[4] Department of Biostatistics, College of Medicine, Pochon CHA University, Kyonggi-do, South Korea.

[5] Biomedlab Research Institute, Seoul, South Korea.

Address for reprints: Hee Jung An, M.D., Department of Pathology, College of Medicine, Pochon CHA University, 351, Yatapdong Bundang-gu Sungnam, Kyonggi-do, 463-712, South Korea; Fax: 011 (82) 317805476; E-mail: hjahn@cha.ac.kr

Received September 9, 2002; revision received November 14, 2002; accepted November 21, 2002.

**BACKGROUND.** Human papillomavirus (HPV) infection is considered to play an important role in the development of cervical carcinoma, and it is known that certain HPV types, such as HPV-16 and HPV-18, are highly associated with cervical carcinoma. However, the pathologic behavior of other HPV types remains unclear. Recently, a new HPV detection technique, the HPV DNA chip, was introduced. The HPV DNA chip harbors 22 HPV probes and has the advantage of being able to detect 22 HPV types simultaneously. To evaluate the quality of the HPV DNA chip method and to identify HPV types related to cervical carcinoma and precancerous lesions, the authors performed HPV typing in cervical specimens from 1983 patients and compared their cytologic and histologic diagnoses.

**METHODS.** The HPV DNA chip was used for HPV typing. Among 1983 patients who were tested for HPV types, cervical smear cytology was performed in 1650 patients, and 677 of those patients underwent cervical biopsy.

**RESULTS.** Among the 1650 smears that were examined cytologically, 92.7% (114 of 123 smears) of low-grade squamous intraepithelial lesions (LSILs), 98.1% (106 of 108 smears) of high-grade squamous intraepithelial lesions (HSILs), and 96.3% (51 of 53 smears) of carcinomas were HPV positive, compared with only 35.1% of smears with normal cytology that were HPV positive. HPV-16 was the most prevalent type (chi-square test; $P < 0.01$) in LSILs (28.5%), in HSILs (51.9%), and in carcinomas (62.5%) followed by HPV-58 and a group of low-risk types (HPV-6, HPV-11, HPV-34, HPV-40, HPV-42, HPV-43,and HPV-44) in LSILs. HPV-58 (15.7%), HPV-18 (6.7%), and HPV-52 (4.6%) were the next most prevalent types after HPV-16 in HSILs. HPV-18 (11.4%) and HPV-58 (11.4%) were the second most common types in carcinomas. HPV-58 had the highest positive predictive value (54.9%) for the detection of histologically confirmed HSIL or carcinoma, whereas HPV 16 had the highest negative predictive value (80.6%). The sensitivity (96.0%) of the HPV test using the DNA chip method for detecting HSIL or carcinoma was superior compared with the sensitivity of cytologic diagnosis (83.6%).

**CONCLUSIONS.** The HPV DNA chip provides a very sensitive method for detecting 22 HPV genotypes with reasonable sensitivity (96.0%) and reasonable negative predictive value (96.9%), and it overcomes the low sensitivity of cytologic screening for the detection of HSIL or carcinoma. HPV-58, HPV-52, and HPV-56, as well as HPV-16 and HPV-18, were associated highly with HSIL and carcinoma in the current large series. In addition, multiple HPV infection was associated less frequently with cervical carcinoma and with precancerous lesions compared with normal cytology. ***Cancer* 2003;97:1672–80.**


**KEYWORDS: human papillomavirus (HPV), DNA chip, cervical carcinoma, squamous intraepithelial lesion.**

Cervical carcinoma is the second most common malignancy in women worldwide,[1] and the role of human papillomavirus (HPV) infection in the development of cervical carcinoma has been well established. Greater than 20 distinct HPV types have been associated with cervical carcinoma.[2] Among these types, it has been found that HPV-16 is the most prevalent type in patients with cervical intraepithelial neoplasia (CIN) and cervical carcinoma, whereas HPV-6 and HPV-11 rarely have been associated with cervical carcinoma. However, the pathologic behaviors of other HPV types remain unclear, and HPV types designated as high-risk still differ from study to study.[3–6] Currently, there seems to be a limitation to cytology-based screening programs in terms of reducing cervical carcinoma incidence and mortality rates. The sensitivity of cytology can be problematic in terms of diagnostic accuracy due to sampling or interpretation errors. It has been reported that the sensitivity of cytology for high-grade CIN was only 40–80%.[7,8] Furthermore, the capability of cytology for detecting endocervical adenocarcinoma is relatively poor, accounting for an increasing incidence in cervical carcinoma. In addition, monitoring for high-risk HPV types has been advocated in women who were treated initially for CIN II or CIN III.[9] Thus, HPV testing is used as a complementary screening method or as an adjunct to conventional cytologic screening.

A new HPV detection technique using a DNA microarray (DNA chip) recently has been developed. This technique is based on the polymerase chain reaction (PCR) method and has the advantage of high sensitivity and the ability to detect single and multiple infection of 22 HPV types at once. By identifying which HPV types are highly associated with cervical precancerous and cancerous lesions, the biologic course of the HPV type specific cervical lesions may be predicted, thus providing appropriate patient care according to HPV type and possibly yielding information that may lead to the development of vaccines for cervical carcinoma.

In this study, we identified HPV types using the HPV DNA chip method in a series of patients with various cervical lesions. HPV type specific cervical lesions and lesion specific HPV types were analyzed to evaluate the quality of the new HPV DNA chip detection method and to assess the oncogenicities of individual HPV types.

## MATERIALS AND METHODS

### Patient Selection

We examined 1983 Korean women who visited the Department of Gynecologic Oncology at Bundang CHA Hospital and who were tested for HPV typing from August 2001 to May 2002. Cervical cytology smears were obtained from 1650 of 1983 patients, and 677 patients underwent cervical biopsy at the same time or within 2 weeks of HPV typing. We compared the diagnoses from cervical cytology smears or the histologic diagnoses with the HPV genotype results. All specimens were used with the approval of the Ethical Committee of Bundang CHA Hospital.

### HPV Genotyping

We used a commercially available HPV DNA chip®, a PCR-based DNA microarray system, as a genotyping method (provided by Biomedlab Company, Seoul, South Korea) and followed the manufacturer's protocol for the assay. The HPV DNA chip contains 22 type specific probes; 15 types from the high-risk group (HPV-16, HPV-18, HPV-31, HPV-33, HPV-35, HPV-39, HPV-45, HPV-51, HPV-52, HPV-56, HPV-58, HPV-59, HPV-66, HPV-68, and HPV-69) and 7 types from the low-risk group (HPV-6, HPV-11, HPV-34, HPV-40, HPV-42, HPV-43, and HPV-44).

Twenty-two type specific 30-mer oligonucleotide probes containing an amine group at the 5′ terminus were immobilized onto a chip slide glass. A slide has four chambers, and each chamber is used for a test. Therefore, a slide tests four samples at one time. Each chamber has two compartments that contain all probes dotted in duplicate; thus, each test is carried out in duplicate for each sample (Fig. 1A-1). Briefly, DNA was isolated from swab samples using a DNA isolation kit (Intron Biotech. Inc., Seoul, South Korea), and target L1 regions of HPV DNA were amplified and labeled by a single dye, indocarbocyanine-dUTP, (NEN® Life Science Products, Inc., Boston, MA), using consensus GPd5+/GP6d+ primers (GP5d+, 5′-tttktachgtkgtdgatacyac-3′; GP6d+, 5′-gaaahataaaytgyaadtcataytc-3′; k, g/t; h, t/a/c; d, a/t/g; y, t/c). $\beta$-Globin was amplified using PCR with PC03/PC04 primers (PC03, 5′-acacaactgtgttcactagc-3′; PC04, 5′-caacttcatccacgttcacc-3′) as internal controls. The PCR products of all samples were detected by electrophoresis through a 2% agarose gel, and the product size of HPV DNA was 150 base pairs (bp). A mixture of 20 $\mu$L of the HPV-amplified product and 10 $\mu$L of the $\beta$-globin-amplified product was denatured by adding 3 N NaOH solution (10% volume/volume), incubating for 5 minutes at room temperature, then neutralized by adding 1 M Tris-HCl, pH 7.2 (5% volume/volume). This was followed with 3N HCl (10% volume/volume) and cooling on ice for 5 minutes. The samples were mixed with a hybridization solution made up of 6 $\times$ saline-sodium phosphate-ethylenediamine tetraacetic acid buffer (SSPE; Sigma, St. Louis, MO) and 0.2% sodium dodecyl sulfate, and then applied onto the DNA chip. Hybridization was performed at 40 °C for 2 hours and was

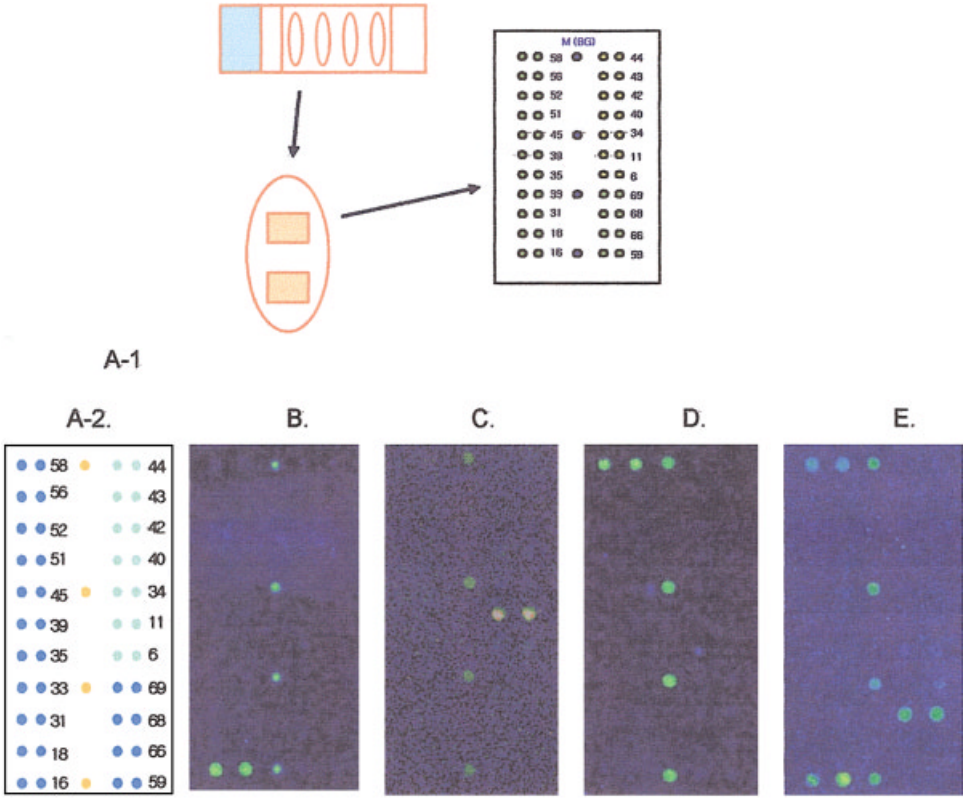


**FIGURE 1.** The human papillomavirus (HPV) DNA chip detection of various HPV types. (A-1) The format of the DNA chip that contains 22 HPV types. Each slide contains two chips that harbor a pair of the oligonucleotide probes of the 22 HPV genotypes. (A-2) Orange dots represent $\beta$-globin, blue dots are probes of 15 high-risk HPV types, and green dots represent probes of 7 low-risk HPV types. (B) Image from an HPV-16 positive patient. (C) Image from an HPV-11 positive patient. (D) Image from an HPV-58 positive patient. (E) Image from a patient who had multiple infection with HPV-16, HPV-58, and HPV-68.

followed by washing with 3 × SSPE for 2 minutes, 1 × SSPE for 2 minutes, and air drying at room temperature.

Hybridized HPV DNA was visualized using a DNA chip scanner (Scanarray lite; GSI Lumonics®, Ottawa, Ontario, Canada). HPV amplicons can be hybridized with corresponding type specific oligonucleotide probe and visualized on HPV DNA chip slides as double positive spots (Fig. 1) when HPV DNA is present in amplified PCR product. The samples that showed a positive band of 150 bp on the gel electrophoresis but were negative on the HPV DNA chip slide were designated as *HPV-other*. DNA either from the HPV-18 positive Hela cell line or from the HPV-16 positive SiHa cell line was used as a positive control in each PCR reaction. None of the negative controls (without DNA) revealed HPV positivity. For slides on which the positive spots were indefinite, the test from the PCR reaction was repeated. An image of an HPV DNA chip showing the various HPV types is illustrated by Figure 1.

### Cytologic and Histologic Diagnosis

Classification of each cytologic diagnosis was based on the Bethesda System. The grading system of low-grade squamous intraepithelial lesion (LSIL) and high-grade squamous intraepithelial lesion (HSIL) also was used for the histologic diagnosis of cervical precancerous lesions to unify the terminology. CIN I and flat condyloma were classified as LSIL, and CIN II and CIN III were classified as HSIL. To avoid interobserver variation, all cytologic and histologic slides were reviewed by two pathologists (H.J.A. and N.H.C.).

### Statistical Analyses

The statistical analyses were performed using SAS software (version 8.0; SAS Institute, Inc., Cary, NC). The chi-square test for equal proportions was used to assess the statistical significance of differences in the prevalence of HPV infection and HPV genotypes by cytologic or histologic diagnoses and to evaluate differences in the frequency of multiple infection among various cervical lesions. To compare the sensitivity, the positive predictive value (PPV), and the negative predictive value (NPV) among HPV types, the $z$ test for comparing two proportions was performed. $P$ values $< 0.05$ were considered statistically significant.

## RESULTS

### Prevalence of HPV Infection

Nine hundred thirteen of 1983 patients (46.04%) were positive for HPV. The mean age of HPV positive patients (42.34 ± 12.03 years) was similar to the mean age of HPV negative patients (41.96 ± 10.55 years). The prevalent types in HPV positive patients are

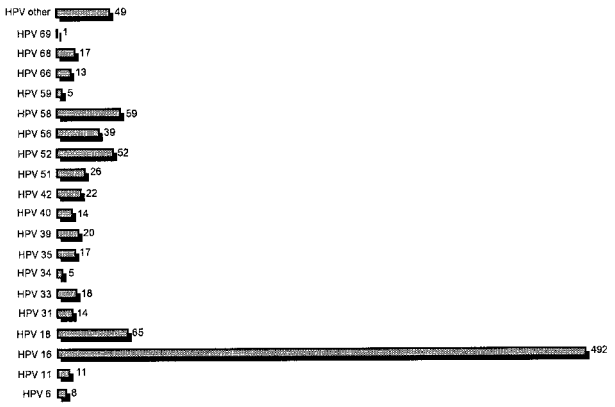


**FIGURE 2.** Number of patients infected with individual human papillomavirus (HPV) types. Each type included single and multiple infection. HPV-16 was the most prevalent type in HPV-infected women (chi-square test; $P < 0.01$) followed by HPV-18, HPV-58, HPV-52, and HPV-56.

shown in Figure 2. HPV-16 was the most prevalent type (492 of 913 patients; 53.8%; chi-square test; $P < 0.01$) followed by HPV-18 (65 of 913 patients; 7.0%), HPV-58 (59 of 913 patients; 6.5%), HPV-52 (52 of 913 patients; 5.7%), and HPV-56 (39 of 913 patients; 4.3%). The prevalence and distribution of HPV genotypes according to cervical cytology results and biopsy results were similar. The prevalence of HPV infection and the 22 HPV genotypes according to cytologic diagnoses are summarized in Table 1. One hundred fourteen patients (92.7%) with LSIL, 106 patients (98.1%) with HSIL, and 51 patients (96.3%) with carcinoma were positive for HPV (chi-square test; $P < 0.01$), compared with 35.1% of patients with normal cytology who had HPV positive results.

Of 677 patients who also underwent cervical biopsy, the results showed that 276 patients had normal histology, 200 patients had LSIL, 151 patients had HSIL, 44 patients had squamous cell carcinoma (SCC), and 6 patients had adenocarcinoma. Seventy-two of 276 patients (26.1%) with normal histology had HPV positive results; whereas 157 of 200 patients (78.5%) with LSIL, 145 of 151 patients (96%) with HSIL, and 48 of 50 patients (96%) with carcinoma had HPV positive results (Table 2).

### Correlations between HPV Genotypes and Precancerous and Cancerous Cervical Lesions

The distribution of prevalent HPV genotypes according to cytologic diagnosis, irrespective of the presence of multiple genotypes, is presented in Table 1. HPV-16 was the most prevalent type (chi-square test; $P < 0.01$) in patients with LSIL (28.5%), HSIL (51.9%), carcinoma (62.5%) followed by HPV-58 and a group of low risk types (HPV-6, HPV-11, HPV-34, HPV-40, HPV-42, HPV-43, and HPV-44) in patients with LSIL. HPV 58 (15.7%), HPV-18 (6.5%), and HPV-52 (4.6%) were the next most common types in patients with HSIL. In patients with carcinoma, HPV-18 (11.4%) and HPV-58 (11.4%) were the second most common types. The prevalence of each HPV type according to histologic diagnosis was similar to the prevalence according to cytologic smear results, as shown in Table 2. HPV-16 and HPV-58 appeared to be associated highly with HSIL, whereas HPV-16, HPV-18, and HPV-58 were more likely to be associated with carcinoma (Fig. 3). Most lesions contained a single HPV genotype, although multiple genotypes (two to five types) were found in a considerable proportion of each cytologic lesion. The frequency of single versus multiple HPV infection, according to cytologic diagnosis, is shown in Figure 4. The detection rate of multiple HPV infection was significantly lower (chi-square test; $P < 0.01$) in patients with LSIL and worse lesions (13.7–20.8%) compared with the detection rate in patients with normal cytology (24.2%). No significant difference was observed between patients with LSIL and patients with HSIL in terms of the frequency of multiple HPV infection.

The PPV of the HPV test for the detection of histologically confirmed HSIL or carcinoma was 45.73%, and HPV-58 showed the highest PPV (54.90%). The NPV was 96.86%, and HPV-16 had the highest NPV (80.63%; $z$ test; $P < 0.05$) among the 22 HPV genotypes (Table 3). The sensitivity for HSIL or worse was 96.02% for any HPV type and 57.21% for HPV-16. This increased to 88.56% when four other prevalent types (HPV-18, HPV-52, HPV-56, and HPV-58) were included. The specificity for HSIL or worse was very high ($z$ test; $P < 0.05$) for HPV-56 (97.69%), HPV-52 (97.27%), HPV-18 (96.64%), and HPV-58 (95.17%) compared with HPV-16 (75.21%). A group of low-risk HPV types accounted for only 8.1% in patients with LSIL; however, those types were not identified in any patients with HSIL or carcinoma. Both the PPV (100%) and the NPV (70.98%) for the detection of LSIL obviously were high for low-risk HPV types (HPV-6, HPV-11, and HPV-34).

### Comparison of Sensitivity of the HPV Test with the Cytologic Smear Results by Reference to Tissue Diagnosis

Among the patients who had their results confirmed by biopsy, many patients with HSIL and carcinoma who were positive for any HPV type with the HPV DNA chip method were under-diagnosed with cytologic smear (Table 4). The HPV DNA chip was positive in 193 of 201 patients with histologically confirmed HSIL and carcinoma, resulting in a sensitivity of 96.0%. The

**TABLE 1**
**Human Papillomavirus (HPV) Genotypes by HPV DNA Chip According to Cervical Cytology**

| | No. of patients (%) | | | | | |
|---|---|---|---|---|---|---|
| HPV genotype | Total | WNL/ reactive | ASCUS/ AGUS | LSIL | HSIL | Ca |
| HPV negative | 829 (50.2) | 742 (64.9) | 74 (33.2) | 9 (7.3) | 2 (1.9) | 2 (4.8) |
| HPV positive | 821 (49.8) | 401 (35.1) | 149 (66.8) | 114 (92.7) | 106 (98.1) | 51 (96.3) |
| 16 | 322 (19.5) | 184 (16.1) | 39 (17.5) | 28 (22.8)[a] | 41 (38.0)[a] | 30 (56.7)[a] |
| Multiple 16[b] | 103 (6.2) | 63 (5.5) | 21 (9.4) | 5 (4.1) | 12 (11.1) | 2 (3.8) |
| 16 and 18 | 22 (1.3) | 9 (0.8) | 7 (3.1) | 2 (1.6) | 3 (2.8) | 1 (2.0) |
| 18[b] | 32 (1.9) | 11 (1.0) | 6 (2.7) | 6 (4.9)[a] | 4 (3.7)[a] | 5 (9.4)[a] |
| 35 | 13 (0.8) | 3 (0.3) | 2 (0.9) | 5 (4.0) | 3 (2.8) | 0 |
| 52[b] | 48 (2.9) | 18 (1.6) | 16 (7.2) | 7 (5.7) | 5 (4.6) | 2 (3.8) |
| 56[b] | 32 (1.9) | 21 (1.8) | 2 (0.9) | 6 (4.9)[a] | 3 (2.8) | 0 |
| 58[b] | 58 (3.5) | 13 (1.1) | 12 (5.4) | 10 (8.1)[a] | 17 (15.7)[a] | 6 (11.4)[a] |
| Other high risk[c] | 109 (6.6) | 34 (3.0) | 25 (11.2) | 31 (25.2) | 15 (13.9) | 4 (7.5) |
| Low-risk type[d] | 42 (2.5) | 28 (2.4) | 4 (1.8) | 10 (8.1) | 0 | 0 |
| Other type[e] | 40 (2.4) | 17 (1.5) | 15 (6.7) | 4 (3.3) | 3 (2.8) | 1 (2.0) |
| Total | 1650 | 1143 | 223 | 123 | 108 | 53 |

HPV: human papillomavirus; WNL: within normal limits; ASCUS: atypical squamous cells of undetermined significance; AGUS: atypical glandular cells of undetermined significance; LSIL: low-grade squamous intraepithelial lesion; HSIL: high-grade squamous intraepithelial lesion; Ca: carcinoma.

[a] Significantly higher than the results for normal cytology and for ASCUS/AGUS in the same row (chi-square test; $P < 0.05$).

[b] Including multiple infection of each type.

[c] Human papillomavirus types 31, 33, 39, 45, 49, 51, 66, 68, and 69.

[d] Human papillomavirus types 6, 11, 34, 40, 42, 43, and 44.

[e] Other type: Human papillomavirus types other than the 22 types found on the DNA chip (positive in polymerase chain reaction analysis but negative on the human papillomavirus DNA chip).

**TABLE 2**
**Human Papillomavirus (HPV) Genotypes by HPV DNA Chip According to Cervical Histology**

| | No. of patients (%) | | | | | |
|---|---|---|---|---|---|---|
| HPV genotype | Total | Reactive | LSIL | HSIL | SCC | AC |
| HPV negative | 255 (37.7) | 204 (73.9) | 43 (21.5) | 6 (4.5) | 2 (4.5) | 0 |
| HPV positive | 422 (62.3) | 72 (26.1) | 157 (78.5)[a] | 145 (96.0)[a] | 42 (95.5)[a] | 6 (100)[a] |
| 16 | 167 (24.7) | 31 (11.2) | 51 (25.5)[a] | 57 (37.8)[a] | 25 (56.8)[a] | 3 (50.0)[a] |
| Multiple 16[b] | 47 (6.9) | 11 (4.0) | 16 (8.0) | 19 (12.6) | 1 (2.2) | 0 |
| 16 and 18 | 18 (2.7) | 2 (0.7) | 6 (3.0) | 7 (4.6) | 2 (4.5) | 1 (16.7) |
| 18[b] | 16 (2.4) | 2 (0.7) | 6 (3.0) | 3 (2.0) | 3 (6.8)[a] | 2 (33.3)[a] |
| 58 | 31 (4.6) | 3 (1.1) | 7 (3.5) | 16 (10.6)[a] | 5 (11.4)[a] | 0 |
| Multiple 58[b] | 6 (0.9) | 0 | 3 (1.5) | 3 (2.0) | 0 | 0 |
| 35 | 11 (1.6) | 2 (0.7) | 4 (2.0) | 5 (3.3) | 0 | 0 |
| 68 | 8 (1.2) | 3 (1.1) | 3 (1.5) | 1 (0.6) | 1 (2.3) | 0 |
| Other high risk[c] | 113 (16.7) | 18 (6.5) | 56 (28.0) | 34 (22.5) | 5 (11.4) | 0 |
| 6, 11, and 34 | 5 (0.7) | 0 | 5 (2.5) | 0 | 0 | 0 |
| Total | 677 (100) | 276 (100) | 200 (100) | 151 (100) | 44 (100) | 6 (100) |

HPV: human papillomavirus; LSIL: low-grade squamous intraepithelial lesion; HSIL: high-grade squamous intraepithelial lesion; SCC: squamous cell carcinoma; AC: adenocarcinoma.

[a] Significantly higher than the results for reactive cervix in the same row (chi-square test; $P < 0.05$).

[b] Including multiple infection of each type.

[c] Human papillomavirus types 39, 45, 51, 52, 56, 59, and 66.

cytologic smear method was less sensitive, with 168 of 201 patients (83.6%) diagnosed as HSIL or carcinoma cytologically. The sensitivity of the HPV test for LSIL (78.5%) was slightly greater compared with the sensitivity of the cytologic smear (64.5%). Among the 33 patients with HSIL who had cytology results of less than HSIL, 30 patients were positive for any HPV type according to the DNA chip method. Therefore, adding the HPV test increased the sensitivity (198 of 201 patients; 98.5%) compared with cytology alone (83.6%) (Table 5).

TABLE 3
**Test Performance of Human Papillomavirus Testing by DNA Chip for the Detection of Histologically Confirmed High-Grade Squamous Intraepithelial Lesion or Worse**

| Characteristic | Any HPV | HPV-16 | HPV-18 | HPV-52 | HPV-56 | HPV-58 | HPV-16, -18, -52, -56, and -58 |
|---|---|---|---|---|---|---|---|
| Sensitivity (%) | 96.02[a] | 57.21[a] | 8.96 | 4.98 | 3.48 | 13.93 | 88.56[a] |
| Specificity (%) | 51.89 | 75.21 | 96.64 | 97.27 | 97.69 | 95.17 | 61.97 |
| PPV (%) | 45.73 | 49.36 | 52.94 | 43.48 | 38.89 | 54.90 | 49.58 |
| NPV (%) | 96.86[a] | 80.63[a] | 71.54 | 70.08 | 70.56 | 72.36 | 92.77[a] |

HPV: human papillomavirus; PPV: positive predictive value; NPV: negative predictive value.
[a] Significantly higher than the results for other types in the same row (Z test; $P < 0.05$).

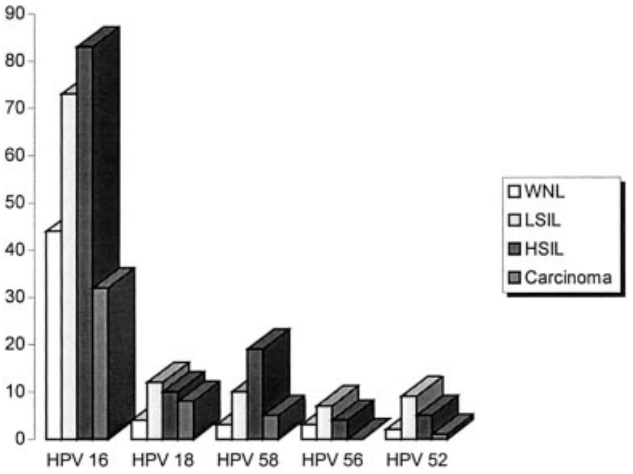


**FIGURE 3.** Number of patients with the five most prevalent human papillomavirus (HPV) types by histologic diagnosis. HPV-16 and HPV-18 were associated most commonly with carcinoma, whereas HPV-16 and HPV-58 more likely were related to high-grade squamous intraepithelial lesion (HSIL). LSIL: low-grade squamous intraepithelial lesion; WNL: within normal limits.

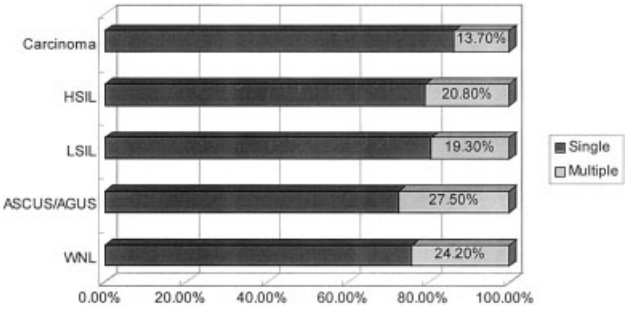


**FIGURE 4.** Frequency of single and multiple human papillomavirus (HPV) infection according to cytologic diagnosis. Multiple HPV infection is less frequent with low-grade squamous intraepithelial lesion (LSIL), high-grade squamous intraepithelial lesion (HSIL), and carcinoma compared with atypical squamous cells of undetermined significance/atypical glandular cells of undetermined significance (ASCUS/AGUS) and cytology within normal limits (WNL) (chi-square test; $P < 0.01$).

## DISCUSSION

It is known that HPV infection is associated with the development of cervical carcinoma and of its precancerous lesion;[4,10,11] therefore, the identification of HPV in the uterine cervix is important clinically. It also is possible to use the HPV test for the detection of cervical lesions as an adjunct to cervical smear cytology. Furthermore, certain HPV types, such as HPV-16 and HPV-18, are related highly with cervical carcinomas and high-grade precancerous lesions. One review article[12] found that HPV-6 and HPV-11 were present in $< 30\%$ of patients with CIN I and that HPV-16 was detected in $> 50\%$ of patients with CIN II and CIN III followed by HPV-31, HPV-33, HPV-35, HPV-39, HPV-45, and HPV-56. However, the causal relation between other HPV types and cervical neoplasia is not understood to date. In the current study, we have presented a new HPV detection technique using a PCR-based DNA microarray system, the HPV DNA chip method, which is devised to detect simultaneously 22 HPV genotypes that have been associated with cervical carcinoma and precancerous lesions. This method is highly sensitive and saves on the time required to run multiple PCR reactions. Therefore, the method provides a convenient way of investigating the relations between the 22 HPV genotypes and the grades of cervical precancerous lesions and carcinomas.

In accordance with many previous studies, HPV-16 was the most prevalent HPV type (Table 1) in patients with HSIL and carcinoma in the current study, and the prevalence of HPV-16 progressively increased with the severity of the cervical lesion; that is, the frequency of HPV-16 was 28.5% in patients with LSIL but 51.9% and 62.5% in patients with HSIL and carcinoma, respectively (chi-square test; $P < 0.05$). In worldwide perspective study of the prevalence of HPV in women with cervical carcinoma,[4] HPV DNA was detected in 93% of tumors, and HPV-16 was present in 50% of these specimens in most countries. HPV-16

**TABLE 4**
**Comparison of Human Papillomavirus Testing and Cytology by Tissue Diagnosis**

| | No. of patients (%) | | |
|---|---|---|---|
| Tissue diagnosis | Total no. | QC cytology category O[a] | HPV positive on DNA chip |
| LSIL | 200 | 129 (64.5) | 157 (78.5) |
| HSIL | 151 | 125 (82.8) | 145 (96.0) |
| SCC | 44 | 39 (88.6) | 42 (95.5) |
| AC | 6 | 4 (66.7) | 6 (100) |
| Total | 677 | 292 | 350 |

QC: quality control; HPV: human papillomavirus; LSIL: low-grade squamous intraepithelial lesion; HSIL: high-grade squamous intraepithelial lesion; SCC: squamous cell carcinoma; AC adenocarcinoma.
[a] Category O (complete agreement) of the quality-control system for cytologic screening (see Travers[24]).

**TABLE 5**
**Sensitivity of Cytology and Human Papillomavirus Testing for the Detection of High-Grade Squamous Intraepithelial Lesion or Worse**

| | HPV test by DNA chip | | |
|---|---|---|---|
| Cytologic diagnosis | No. negative | No. positive | Total no. (%) |
| < HSIL | 3 | 30 | 33 (16.4) |
| ≥ HSIL | 5 | 163 | 168 (83.6) |
| Total no. (%) | 8 (4.0) | 193 (96.0) | 201 (100) |

HPV: human papillomavirus; HSIL: high-grade squamous intraepithelial lesion.

was followed by HPV-18 (13.7%), HPV-45 (8.4%), and HPV-31 (5.3%). However, the second most common types in the current study were HPV-18 and HPV-58 (11.4%), and HPV-31 and HPV-45 were not detected in patients with carcinoma. The prevalence of HPV-16 in patients with HSIL in the current study (51.9%) was comparable to its prevalence in other previous reports (43–72%) that used the PCR method.[13–15] The next most common types in this study differed slightly from those reported in other studies. HPV-58, in addition to HPV-18, was the second most common type, whereas HPV-31 and HPV-35 were more frequent in other studies.[14–16] However, a more recent study using a PCR/reverse blot-strip assay[17] reported that HPV 50s, including HPV-51, HPV-52, HPV-56, and HPV-58, were the most common HPV types after HPV-16 in patients with dysplasia. Therefore, it is likely that the differences between studies with respect to the second most common types depends on the methods used rather than any ethnic or geographic variations.

Although HPV-16 is the most prevalent type worldwide, the PPV of HPV-16 for HSIL or carcinoma (49.36%) is lower than that of HPV-58 (54.90%), because HPV-16 is the most prevalent type not only in HSIL or carcinoma but also in LSIL and in normal cytology. Meanwhile, the NPV of HPV-16 is highest (80.63%; $z$ test; $P < 0.05$) among the prevalent HPV types (HPV-16, HPV-18, HPV-52, HPV-56, and HPV-58). When adding the other prevalent types (HPV-18, HPV-52, HPV-56, and HPV-58) to HPV-16, the PPV and NPV are improved reasonably to 49.58% and 92.77%, respectively.

LSIL originally was associated with low oncogenic risk HPV.[18] However, LSIL has been related to heterogeneous HPV types in several recent studies,[13,19] which have shown an association with low-risk HPV types in only 15% of patients with LSIL. In the current study, HPV-16 was the most prevalent type in patients with LSIL (28.5%) and in patients with HSIL and carcinoma. The frequency of low-risk HPV types in patients with LSIL was only 8.1%. Thus, the results available to date indicate that the low-risk HPV types are associated infrequently with LSIL, and various high-risk HPV types (HPV-16, HPV-18, HPV-31, HPV-33, HPV-35, HPV-45, HPV-52, HPV-56, HPV-58, HPV-66, and HPV-68) are related to LSIL. Conversely, the low-risk HPV types were never seen in patients with HSIL or carcinoma in this study.

The association between multiple HPV infection and the development of cervical neoplasia and carcinoma remains controversial, although some previous reports[17] have indicated that multiple HPV infection contributes to the development of cervical neoplasia. The incidence of infection by two or more different HPV types reportedly is 10–20%[19–22] in HPV-infected women. This variability probably is due to population or age differences and the methods adopted. One study of 180 women with low-grade dysplasia[23] found that 58.9% of patients had between 2 and 6 different HPV types. Another PCR-based study[17] demonstrated a mean of 3.29 different HPV types detected in patients with cervical dysplasia compared with 1.04 HPV types in patients with normal cytology. These results support the notion of a possible role for multiple HPV types in the development or progression of cervical dysplasia. The overall frequency of multiple infection in the current study was 23.0%. However, our study produced different results with respect to the multiplicity of HPV infection in patients with cervical neoplasia. The incidence of multiple infection in cancerous and in precancerous lesions was significantly lower compared with the incidence in specimens with normal cytology (chi-square test; $P < 0.01$). We believe that the method used caused the difference between the current results and the results of other investigators. The HPV DNA chip is very sensitive method that can detect even 18 fg of HPV DNA, which makes it

much more sensitive compared with any other PCR-based method. Thus, the DNA chip method is more sensitive for the detection of multiple HPV infection in specimens with normal cytology, in which the amount of each type of HPV DNA may be very small. In view of the fact that the current study represents a large series, we suggest that multiple infection is associated less frequently with cervical neoplasia than the normal cervix and the reactive cervix (chi-square test; $P < 0.01$).

For patients with histologically confirmed results, the cytologic test for cervical lesions from LSIL to carcinoma showed a sensitivity of 64.5–88.6% when Category O (complete agreement) was adopted from a quality-control system.[24] In contrast, the HPV DNA chip method showed greater sensitivity of 78.5–96.0%. Sensitivity for detecting HSIL and carcinoma was improved significantly when the HPV DNA chip test was added to the cytologic test. Among 33 patients with HSIL or carcinoma who were under-diagnosed using the cytologic smear method, 30 patients were identified as HPV positive (Table 5). Thus, the overall sensitivity for detecting HSIL and carcinoma would be increased to 98.5%. A combination of the cytologic test and the HPV DNA chip test yielded an NPV of 98.8% for the detection of HSIL and carcinoma. These results support the notion that the HPV DNA chip test can overcome the lower sensitivity of cytologic screening for the detection of HSIL or worse. Moreover, the sensitivity of the HPV DNA chip test for detecting histologically diagnosed HSIL or carcinoma (96.02%) is as high as that of the previously reported hybrid capture II assay (78–95%).[25] The specificity of the HPV DNA chip method for detecting HSIL or carcinoma was 51.89%; however, this increased to 73.91% when LSIL was included. The NPV of the HPV DNA chip method (96.86%) was comparable of the NPV of the hybrid capture II assay (98.9%), and the PPV of the HPV DNA chip method (45.73%) was greater compared with the PPV of the hybrid capture II assay (10.0–19.6%). Compared with two German studies[26,27] that used GP5+/GP6+ PCR and hybrid capture I, the sensitivity and PPV of the HPV DNA chip test in the current study (96.0% and 45.7%, respectively) were superior to their results (89% and 35.8%, respectively).

In the current study, the HPV DNA chip proved to be a very sensitive method for detecting the 22 HPV genotypes. It had reasonable PPV and NPV, and it substantially overcame the low sensitivity problem of cytologic screening for the detection of HSIL or carcinoma. HPV-58, HPV-52, and HPV-56, as well as HPV-16 and HPV-18, were associated highly with HSIL and carcinoma in the current large series. In addition, multiple HPV infection was associated less frequently with cervical carcinoma and precancerous lesions compared with normal cytology.

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