Evaluation of malignant potential based on infection with high-risk human papillomaviruses 16, 52, and 58 in the uterine cervix

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Article

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Abstract

High-risk human papillomavirus (HR-HPV) is known as the most important carcinogen in uterine cervical carcinoma. Previous studies have evaluated genotype-specific risk for carcinogenesis. However, the genotype-specific risk remains still unclear due to some limitations of those studies. This study aimed to evaluate the malignant potential of the three most prevalent HR-HPVs in Korea. Patients who underwent cervical conization were included. They had received HPV test within a year before the surgery and those exhibiting concurrent multiple infections with HR-HPVs were excluded. Of single infections with HR-HPV, the three most prevalent HR-HPVs were included to analyze. To evaluate their malignant potential, CIS+, including carcinoma in situ (CIS) and invasive carcinoma, was categorized in each HR-HPV group. The ratios of pathologic diagnoses and odds ratios for malignant potential were evaluated between the three most prevalent HR-HPVs. Totally 230 patients were found to have a single infection with HR-HPV16, HR-HPV52, or HR-HPV58. The HPV16 group did not exhibit a significantly more CIS, invasive carcinoma, and CIS+ than HPV52 or HPV58. Physicians should pay attention to not only HPV16 but also HPV52 and HPV58 because these genotypes have similar malignant potential. These findings support the need for a nine-valent vaccine against HR-HPVs in Korea.

Introduction

Uterine cervical cancer including squamous cell carcinoma and adenocarcinoma is the fourth most common cancer worldwide\(^1\) and the fifth one in women in South Korea\(^2\). High-risk human papillomavirus (HR-HPV) is a critical carcinogen in uterine cervical carcinoma, and persistent infection with this virus accounts for 90% of all cervical cancer incidences\(^3\)\(^-\)\(^5\). HPV has over 100 genotypes, with approximately 20 of them recognized as high-risk genotypes\(^5\)\(^-\)\(^8\). HR-HPVs have been reported to have different distributions according to geographical regions or countries; however, in general, HPV16 and HPV18 are the two most prevalent worldwide\(^8\)\(^-\)\(^16\). However, whether the malignant potential of each HR-HPV is similar or significantly different remains unclear.

HPV infects the basal layer of the squamous epithelium and causes malignant progression with alterations of various signaling pathways in the uterine cervix\(^7\)\(^,\)\(^16\). Among HPV oncogenes, E6 and E7 play a significant role in HPV-induced carcinogenesis\(^17\). The E6 viral protein binds to p53 and inhibits its function via ubiquitin-dependent degradation, whereas the E7 protein promotes cell proliferation via pRB inhibition\(^18\)\(^,\)\(^19\). Genomic instability and tumor-promoting host cell mutations are induced by the function of these proteins and their interactions with other signaling molecules\(^7\).

Previous studies have evaluated the malignant potential of single infection with HR-HPV\(^10\)\(^,\)\(^11\). However, these studies have some limitations owing to the obscure exclusion criteria. Furthermore, in these studies, it remains unclear whether the authors excluded the patients with multiple HR-HPV infections.

This study aimed to evaluate the malignant potential of the three most prevalent HR-HPVs in Korea with thorough control of clinical factors.

Methods

Patients. We retrospectively reviewed 755 patients who underwent uterine cervical conization for the diagnosis or treatment of cervical pre-malignant lesions or carcinoma from January 2012 to February 2023 in Kyungpook National University Hospital (KNUH). Sixty-two patients were excluded owing to the absence of HPV test within 1 year preoperatively. Additionally, 96 patients were excluded owing to the negative HR-HPV result on the HPV test. One woman was excluded because of her medical history of ovarian cancer. We excluded 229 patients because of concurrent multiple HR-HPV infections, two or more HR-HPV genotypes, on the test. Of the patients who appeared to exhibit a single HR-HPV infection, we included the three most prevalent HR-HPV genotypes. The flow diagram for patient selection is presented in Fig. 1. Informed consent was waived because of the retrospective nature of the study and the analysis used anonymous clinical data. All methods in this study were in accordance with the Animal Research: Reporting of In Vivo Experiments guidelines 2.0. and the Declaration of Helsinki. This study was approved by the Institutional Review Board of KNUH (KNUH 2023-06-015).

Definition of initial and final diagnoses. The initial diagnosis was determined on the basis of the pathologic result of cervical swab cytology, the Pap smear or liquid-based thinPrep, along with the 2001 Bethesda System or cervical biopsy\(^20\). Inflammation or reactive change, or benign cervical polyp was classified as normal. The atypical cells included atypical squamous cells (ASCs), ASC of undetermined significance, ASC cannot exclude high-grade lesion, atypical glandular cells (AGC), and AGCs of undetermined significance. The final diagnosis was determined on the basis of the pathologic result of uterine cervical conization. Acute or chronic inflammation was classified as normal. In the initial or final diagnosis, the low-grade squamous intraepithelial lesion (LSIL) included koilocytosis without definite dysplasia, or dysplasia for which the grade cannot be determined, not only cervical intraepithelial neoplasm (CIN) 1. The high-grade squamous intraepithelial lesion (HSIL) included CIN2 or CIN3; however, it did not include carcinoma in situ (CIS). Instead, the carcinoma in the initial diagnosis and the CIS in the final
diagnosis included these CIN3 cases, which had been diagnosed with CIS. In the initial diagnosis, as the invasiveness was obscure in some cases, the carcinoma included CIS and invasive carcinoma. When lesions of two different grades such as LSIL and HSIL were simultaneously detected, the diagnosis of higher malignant potential was made.

**Classification of carcinoma in situ + (CIS+).** To evaluate the malignant potential between each group in the three most prevalent single HR-HPV infection groups, we categorized a subgroup of CIS+. It included CIS and invasive carcinoma, regardless of the histologic subtype, such as squamous cell carcinoma or endocervical adenocarcinoma.

**Statistical analysis.** Categorical variables were evaluated using the Chi-square test or Fisher’s exact test, whereas continuous variables were compared using one-way analysis of variance and the Scheffe test for *post-hoc* analysis. Logistic regression was used to analyze the correlation of the HSIL + or CIS + ratios in the three most prevalent HR-HPV genotypes. A *P*-value of < 0.05 was considered statistically significant. All statistical analyses were performed using Statistical Package for the Social Sciences version 26 (IBM Corp., Armonk, NY, USA).

**Uterine cervical conization.** The loop electrosurgical excision procedure was adopted to perform uterine cervical conization. In this study, a total of 15 surgeons performed this surgery. According to the decision of the surgeon, endocervical conization and/or endocervical curettage were also performed. To determine the resection margin, colposcopy using acetic acid was employed.

**HPV test.** The sample for the HPV test was harvested using a uterine cervical swab. This sample was sent to a pathologist for the test via a liquid medium. To determine the HPV infection status, two different methods were employed, including DNA microarray and real-time polymerase chain reaction (RT-PCR), owing to their similar validation. The HPV tests were not centrally reviewed, and the titer of HPV DNA was not taken into consideration. Thus, the results in local medical institutions, not only in our institution, were also included in this study.

**Results**

Of the included patients, 119 (51.7%), 60 (26.1%), and 51 (22.8%) were noted to be infected with HR-HPV genotypes 16, 52, and 58, respectively. Furthermore, the mean ages of the patients were 45.38 ± 12.87, 48.42 ± 12.46, and 45.94 ± 12.89 years, respectively, indicating no significant difference (*p* = 0.317). No significant difference was observed in the distribution between the initial diagnosis, except the carcinoma, based on cervical biopsy or cervical swab cytology. Each of the HPV16 and HPV58 single infection groups showed significantly more carcinoma cases than the HPV52 single infection group (HPV16 vs. HPV52, *p* = 0.006; HPV58 vs. HPV52, *p* = 0.032; HPV16 vs. HPV58, *p* = 0.864) (Table 1).
Table 1
Characteristics and clinical factors of patients with a single HR-HPV genotype infection. The initial diagnosis is determined on the basis of cervical swab cytology or punch biopsy.

<table>
<thead>
<tr>
<th></th>
<th>Single infection group with HPV 16 (n = 119)</th>
<th>Single infection group with HPV 52 (n = 60)</th>
<th>Single infection group with HPV 58 (n = 51)</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>45.38 ± 12.87</td>
<td>48.42 ± 12.46</td>
<td>45.94 ± 12.89</td>
<td>0.317</td>
</tr>
<tr>
<td>Initial diagnosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>1 (0.8%)</td>
<td>0 (0%)</td>
<td>1 (2.0%)</td>
<td>0.540</td>
</tr>
<tr>
<td>Atypical cells</td>
<td>9 (7.6%)</td>
<td>9 (15.0%)</td>
<td>4 (7.8%)</td>
<td>0.250</td>
</tr>
<tr>
<td>LSIL</td>
<td>10 (8.4%)</td>
<td>9 (15.0%)</td>
<td>5 (9.8%)</td>
<td>0.390</td>
</tr>
<tr>
<td>HSIL†</td>
<td>52 (43.7%)</td>
<td>31 (51.7%)</td>
<td>22 (43.1%)</td>
<td>0.552</td>
</tr>
<tr>
<td>Carcinoma‡</td>
<td>47 (39.5%)</td>
<td>11 (18.3%)</td>
<td>19 (37.3%)</td>
<td>0.015§</td>
</tr>
</tbody>
</table>

Data are expressed as numbers (%) and means ± standard deviations.

*: Statistical significance is evaluated using one-way analysis of variance with post-hoc analysis in the age and Chi-square test in the initial diagnosis.

†: Includes CIN2 and CIN3, which is not carcinoma in situ.

‡: Includes CIS, invasive carcinoma, and some CIN3, which was diagnosed with the CIS.

§: HPV16 vs. HPV52, P = 0.006, HPV16 vs. HPV58, P = 0.864, HPV52 vs. HPV58, P = 0.032.

Abbreviations
LSIL, low-grade squamous intraepithelial lesion; HSIL, high-grade squamous intraepithelial lesion; CIS, carcinoma in situ; CIN, cervical intraepithelial neoplasm

Final diagnoses, which were determined through a pathological examination of the cervical conization, were also compared among the three groups. Unlike the initial diagnoses, no significant differences were noted in the distribution of the final diagnoses. HSIL was diagnosed in 19 (16.0%), 14 (23.3%), and 10 (19.6%) patients in the HPV16, HPV52, and HPV58 single infection groups, respectively (p = 0.482). CIS was diagnosed in 59 (49.6%), 27 (45.0%), and 25 (49.0%) patients in the HPV16, HPV52, and HPV58 single infection groups, respectively (p = 0.839). Invasive carcinoma, regardless of histology such as squamous cell carcinoma or adenocarcinoma, was diagnosed in 8 (6.7%), 1 (1.7%), and 1 (2.0%) patient in the HPV16, HPV52, and HPV58 single infection groups, respectively (p = 0.187). The CIS + ratio was not significantly different among the HPV16, HPV52, and HPV58 single infection groups (67 [56.3%] vs. 28 [46.7%] vs. 26 [60.0%], p = 0.460) (Table 2).
Table 2
Comparison of final diagnoses among the high-risk HPV16, HPV52, and HPV58 single infection groups. Final diagnosis is determined on the basis of the pathologic examination following uterine cervical conization.

<table>
<thead>
<tr>
<th></th>
<th>Whole group</th>
<th>Subgroup (≤ 50 years)</th>
<th>Subgroup (&gt; 50 years)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HPV16</td>
<td>HPV52</td>
<td>HPV58</td>
</tr>
<tr>
<td></td>
<td>(n = 119)</td>
<td>(n = 60)</td>
<td>(n = 51)</td>
</tr>
<tr>
<td>Normal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>19 (16.0%)</td>
<td>10 (16.7%)</td>
<td>6 (11.8%)</td>
</tr>
<tr>
<td></td>
<td>13 (16.5%)</td>
<td>5 (6.3%)</td>
<td>6 (15.8%)</td>
</tr>
<tr>
<td></td>
<td>16 (20.3%)</td>
<td>9 (23.7%)</td>
<td>9 (28.1%)</td>
</tr>
<tr>
<td></td>
<td>42 (53.2%)</td>
<td>19 (50.0%)</td>
<td>14 (43.8%)</td>
</tr>
<tr>
<td></td>
<td>8 (12.5%)</td>
<td>1 (4.5%)</td>
<td>1 (5.3%)</td>
</tr>
<tr>
<td></td>
<td>67 (56.3%)</td>
<td>28 (46.7%)</td>
<td>26 (60.0%)</td>
</tr>
</tbody>
</table>

Data are expressed as numbers (%).

*: All statistical significances are evaluated using the Chi-square test.

†: Includes CIN2 and CIN3, which is not carcinoma *in situ*.

‡: Includes CIS and invasive carcinoma, and some CIN3, which was diagnosed with the CIS.

§: Includes CIS and invasive carcinoma.

Abbreviations
LSIL, low-grade squamous intraepithelial lesion; HSIL, high-grade squamous intraepithelial lesion; CIS, carcinoma *in situ*; CIN, cervical intraepithelial neoplasia

In the logistic regression analyses, in the evaluation of the malignant potential (CIS+), no significant difference was observed between the three single HR-HPV infection groups. Between the HPV16 and HPV52 single infection groups, the odds ratio (OR) for CIS+ was 0.679 (95% confidence interval [CI] = 0.364–1.267, \( p = 0.224 \)). Between the HPV16 and HPV58 single infection groups, the OR for CIS+ was 0.807 (95% CI = 0.418–1.558, \( p = 0.523 \)). Between the HPV52 and HPV58 single infection groups, the OR for CIS+ was 1.189 (95% CI = 0.563–2.510, \( p = 0.651 \)).

Discussion

Although HR-HPV16 was the most prevalent genotype among Korean women, it exhibited a similar malignant potential to HR-HPV52 and HR-HPV58 in the uterine cervix. Additionally, the risk of requiring medical intervention did not significantly differ among the three single infection groups.

The results of this study can help physicians explain the malignant potential of HR-HPV genotypes 16, 52, and 58 to their patients. HR-HPV16, the most prevalent genotype in Korea, did not exhibit a significantly higher risk of carcinogenesis in the uterine cervix than HR-HPV52 and HR-HPV58, which are the second and third most prevalent genotypes, respectively. Physicians should explain that the malignant potential of HPV52 or HPV58 is not inferior to that of HPV16. Furthermore, as the quadrivalent vaccine does not cover HPV52 or HPV58, our results support the need for a nine-valent vaccine against HR-HPVs, covering genotypes 6, 11, 16, 18, 31, 33, 45, 52, and 58.

To evaluate the malignant potential while controlling for age, subgroup analysis was performed. The entire patient cohort was divided into two subgroups on the basis of age, one with individuals aged 50 years or younger and the other with individuals older than 50 years. In the subgroup of individuals aged 50 years or younger, the number of HPV16, HPV52, and HPV58 single infection groups were 79 (53.0%), 38 (25.5%), and 32 (21.5%) cases, respectively. The prevalence of CIS+ was observed in 45 (57.0%), 19 (50.0%), and 14 (43.8%) cases, respectively. In this subgroup, the CIS+ ratios were not significantly different (\( p = 0.426 \)). Logistic regression did not show significant ORs between any of the three single infection groups. Specifically, for HPV16 versus HPV52, HPV16 versus HPV58, and HPV52 versus HPV58, the
ORs were 0.756 (95% CI = 0.348–1.642, p = 0.479), 0.588 (95% CI = 0.257–1.345, p = 0.208), and 0.778 (95% CI = 0.302–2.000, p = 0.602), respectively. In the subgroup of individuals older than 50 years, 40 (49.4%), 22 (27.2%), and 19 (23.5%) cases of HPV16, HPV52, and HPV58, respectively, were noted. The prevalence of CIS+ was observed in 22 (55.0%), 9 (40.9%), and 12 (63.2%) cases, respectively. Similar to the subgroup of individuals aged 50 years or younger, no significant differences were noted in the CIS+ ratio, and the risk of CIS+ was not significantly different in the logistic regression test (HPV16 vs. HPV52, OR = 0.566 [95% CI = 0.197–1.625, p = 0.290]; HPV16 vs. HPV58, OR = 1.403 [95% CI = 0.457–4.304, p = 0.554]; HPV52 vs. HPV58, OR = 2.476 [95% CI = 0.701–8.742, p = 0.199]) (Table 2).

In Tables 1 and 2, we identified a discrepancy between the ratios of HSIL and CIS in the initial and final diagnoses within all three single infection groups. As mentioned in the Methods section, the initial diagnosis was determined on the basis of cervical swab cytology or punch biopsy in the office. Therefore, we assessed the diagnostic accuracy of cervical swab cytology and punch biopsy under colposcopy with acetic acid application to detect HSIL, CIS, or invasive carcinoma. All patients, regardless of their HPV infection status or whether they had undergone an HPV test within 1 year before conization, were included in this analysis, except for patients without cervical swab cytology or biopsy under colposcopy results. The pathologic diagnoses of both screening tests and cervical conization were categorized into HSIL+ and HSIL−. HSIL+ encompassing normal, atypical cells, and LSIL, whereas HSIL− included HSIL, CIS, and invasive carcinoma.

In the cervical swab cytology for detecting HSIL+ (n = 738), the sensitivity (SS), specificity (SP), positive predictive value (PPV), and negative predictive value (NPV) was 32.8% (150/458), 86.1% (241/280), 79.4% (150/189), and 43.9% (241/549), respectively. The overall diagnostic accuracy was 53.0% (391/738). In the cervical punch biopsy under colposcopy with acetic acid application for detecting HSIL+ (n = 597), the SS, SP, PPV, and NPV was 89.5% (365/408), 42.3% (80/189), 77.0% (365/474), and 65.0% (80/123), respectively. The overall diagnostic accuracy was 74.5% (445/597).

Based on some recent studies, the Pap smear appeared to have a sensitivity of 47.2–55.5% and a specificity of 64.8–75.0%, whereas cervical biopsy under colposcopy showed a sensitivity and specificity of 64.7% and 52.74%, respectively. Considering our data, cervical swab cytology and colposcopy examination appear to offer the benefits of high specificity and high sensitivity, respectively. However, as these examinations can exhibit low reproducibility, further studies are needed to establish optimal guidelines, especially in limited or resource-constrained conditions.

The count and ratio of each HR-HPV genotype including all genotypes not only HPV16, 52, and 58 among the patients enrolled in this study are presented in Fig. 2. All subgroups represent the single infection group. The 10 most prevalent genotypes, in descending order, were HPV16, 52, 58, 18, 33, 31, 51, 53, 56, and 66.

Recent studies have analyzed the HPV genotype-specific risk for carcinogenesis in the uterine cervix. Park E et al. evaluated the risk on the basis of cervical biopsy. In this study, we identified HPV16, HPV52, and HPV58 as the three most common HR-HPV genotypes in Korean women, which is consistent with the reports of those authors. However, unlike the results of their study, our results were based on the pathologic results of uterine cervical conization. Moreover, they did not elucidate whether the patients infected with multiple HR-HPV genotypes were excluded. Thus, although they demonstrated a significantly higher carcinogenic risk for several HR-HPV genotypes including HPV16, HPV52, and HPV58 and a much higher OR of HPV16 than other genotypes, multiple infections with HR-HPV genotypes may have influenced these results. In this study, the HR-HPV genotype 16-, 52-, and 58-specific risk for carcinogenesis was clearly shown owing to the exclusion of concurrent multiple HR-HPV infections. On the basis of age, the authors stratified the patients into the following five subgroups: ≤34, 35–44, 45–54, 55–64, and ≥65 years. This enabled them to evaluate age- and HR-HPV genotype-specific risks.

Another similar study conducted by So KA et al. was based on cervical biopsy results. They did not describe the inclusion or exclusion criteria for age; based on the result, the authors seemed to have included patients of all ages. In their study, HR-HPV genotypes 16, 52, and 58 were the most prevalent, which is consistent with our results. Their results showed that some HR-HPV genotypes such as 16, 31, 33, 52, and 58 were significantly more in CIN2, CIN3, and cervical cancer than those in the normal or CIN1 group. However, as patients with concurrent multiple infections were included in their study, the genotype-specific risk was unclear.

The strength of this study was the homogeneous cohort with a single infection of HPV16, HPV52, or HPV58. This enabled the study to evaluate the malignant potential of each genotype with less bias compared with previous studies.

This study had some limitations. First, this was a retrospective study with a small sample size from a single center. Owing to the small sample size, the cohort could not be further stratified on the basis of age. Therefore, bias may arise from the different sexual activities according to age. A selection bias might also have affected the results because some patients, postmenopausal or not wanting more pregnancies, could have chosen to receive hysterectomy due to HSIL, CIS, or microinvasive carcinoma rather than cervical conization. Second, several patients in the cohort did not undergo follow-up HPV test before surgery. Thus, we could not prove or control the influence of persistent HR-HPV infection despite it being known as a critical factor in cervical cancer. Third, we could not review the HPV vaccination history. The
Korean government included the quadrivalent and bivalent HPV vaccines (against HR-HPV6, 11, 16, and 18; against HR-HPV16 and 18) in the National Program 2016 and provided these vaccines for girls aged 12–17 years. According to statistics from the Korean government, approximately 70% of Korean girls have received the vaccines\(^2\). Finally, the HPV test in this study included two different methods, including DNA microarray and RT-PCR. Heterogeneity might have arisen from this aspect, raising concerns about the reliability of detecting HPV genotypes. When the titration of a certain HPV DNA was very high, other HPV genotypes’ DNA could not have been detected on the HPV test (references?? Detection accuracy??). Some patients could have had concurrent multiple infection of HR-HPVs, even though they had been shown to have a single HR-HPV infection on the HPV test. Furthermore, the HPV test was not centrally reviewed.

**Conclusion**

Among the Korean women with single HR-HPV infection, HPV16, HPV52, and HPV58 were the three most common genotypes, in descending order. Although HPV16 was the most prevalent among the three, its malignant potential was not significantly different from those of HPV52 and HPV58 in the uterine cervix. This result supports the need for a nine-valent vaccine against HR-HPVs, covering HPV52, HPV58, and HPV16.

**Declarations**

**Competing interests**

The authors declare no competing interest.

**Author Contribution**

J Lee and HJ Lee performed data curation. J Lee wrote the main manuscript and prepared tables and figures. HJ Lee supervised the study. All authors reviewed the manuscript.

**Data availability**

The data that support the findings of this study are available on reasonable request from the corresponding author.

**References**


Figure 1

Flow diagram for patient selection
Figure 2

Graph presenting the count and ratio of each HR-HPV genotype, including all genotypes not only HPV16, 52, and 58, among the patients enrolled in this study. All subgroups represent the single infection group.