임상연구 계획 및 논문 작성의 실제 Ⅱ

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강 우 대
Human papillomavirus genotyping as a reliable prognostic marker of recurrence after loop electrosurgical excision procedure for high-grade cervical intraepithelial neoplasia (CIN2-3) especially in postmenopausal women

Woo Dae Kang, MD, PhD, and Seok Mo Kim, MD, PhD

Abstract

Objective: This study was conducted to determine, using the HPV DNA Chip (HDC) test, whether the human papillomavirus (HPV) genotype is predictive of recurrent high-grade cervical intraepithelial neoplasia (CIN; CIN2-3) after a loop electrosurgical excision procedure (LEEP) in postmenopausal women.

Methods: Between January 2007 and February 2013, 206 postmenopausal women with CIN2-3 were treated with LEEP, followed by cytology, Hybrid Capture II (HC2) assay, and HDC test. Post-LEEP follow-up was performed at 3, 6, 9, 12, 18, and 24 months during the first 2 years and yearly thereafter.

Results: Among 206 women, HC2 yielded positive results in 199 women (96.6%) and HDC yielded positive results in 201 women (97.6%) before LEEP. The overall agreement between HDC and HC2 was 99.0%. The area under the receiver operating characteristic curve for high-risk HPV (HR-HPV) viral load measured by HC2 predicting recurrent CIN2-3 was 0.567 ($P = 0.335$). Twenty-six women (12.6%) developed recurrence, and those who developed recurrence tested positive for the same HR-HPV genotype before and after LEEP. The same HR-HPV genotype by HDC during follow-up had a sensitivity and negative predictive value of 100% in detecting recurrent disease. HPV-18 was significantly associated with recurrent CIN2-3 ($P < 0.05$).

Conclusions: Among postmenopausal women, persistent infection with the same HR-HPV genotype, especially HPV-18, should be considered a risk factor for developing recurrent CIN2-3. After LEEP, such women warrant special attention with intense follow-up.

For example, in the most recent Journal Citation Report (2014), Menopause ranked 10th out of the 79 journals listed in the Ob/Gyn category with an impact factor of 3.361.
Introduction

*Gynecologic Oncology,* an international journal, is devoted to the publication of clinical and investigative articles that concern tumors of the female reproductive tract. We welcome the submission of investigations relating to the etiology, diagnosis, treatment, and prevention of female cancers, as well as research from any of the disciplines related to this field of interest. Research areas include: cell and molecular biology, chemotherapy, clinical trails, epidemiology, genetics, immunology and vaccines, 'omics', pathology and cytology, quality of life, radiation therapy, surgery, and translational research. All aspects of scholarship related to tumors of this region are welcome, with **originality, quality, and clarity** the chief criteria of acceptance.
What’s the originality?

HPV AND CERVICAL NEOPLASIA
# US FDA-approved HPV tests

<table>
<thead>
<tr>
<th>Name</th>
<th>Company</th>
<th>HPV Gently Detection</th>
<th>Uses</th>
<th>FDA approval date</th>
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</table>
| Digene Hybrid Capture 2 High-Risk HPV DNA Test | QIAGEN, Germantown, MD        | 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68     | 1. ASC-US triage  
2. Co-testing in women >30 years old                                                                 | March, 2003       |
| Cervista HPV HR                           | Hologic, Bedford, MA           | 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68 | 1. ASC-US triage  
2. Co-testing in women >30 years old                                                                 | March, 2009       |
| Cervista HPV 16/18                        | Hologic, Bedford, MA           | 16, 18                                                      | 1. Triage for follow up of women >30 years old with negative cytology and positive high-risk HPV.               | March, 2009       |
| Cobas HPV Test                            | Roche Molecular Systems, Pleasanton, CA | Specifically identities 16 and 18 while concurrently testing for 31, 33, 35, 39, 51, 52, 45, 56, 58, 59, 66, and 68 | 1. ASC-US triage  
2. mCo-testing in women >30 years old  
3. Triage for follow up of women >30 years old with negative cytology and positive high-risk HPV testing | April, 2011       |
| APTIMA HPV Assay                          | Gen-Probe, San Diego, CA       | 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68 | 1. ASC-US triage  
2. Co-testing in women >30 years old                                                                 | February, 2012    |

MyHPV Chip kit (MyGene, Co)
Detection of HPV genotypes in cervical lesions by the HPV DNA Chip and sequencing

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MyGene Bioscience Institute, MyGene Company, Seoul, Republic of Korea

Received 23 February 2005
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Abstract

Objective. A newly introduced HPV detection technique in cervical lesion, the HPV DNA Chip test, contains 24 HPV probes and has the advantage of being able to detect 24 HPV types at once. We performed HPV DNA sequencing and compared the results with that of the HPV DNA Chip for evaluation of the accuracy of the DNA Chip test.

Methods. The HPV DNA sequencing was performed in samples of 282 patients, where specific HPV type had been shown in HPV DNA Chip test. The sixteen cases where multiple HPV types had been found in HPV DNA Chip test were included in 282 cases. The sequencing was also performed in HPV-chip other type samples of 95 patients, where specific HPV type had been shown in HPV DNA chip test.

Results. In 257 cases (91.1%) of 282 cases, the HPV types of the HPV DNA sequencing test were in agreement with types of the HPV DNA Chip. In 16 cases (5.7%), the sequencing types were different from types of HPV DNA Chip. But in 9 of 16 cases, types in HPV DNA sequencing were absent types in HPV DNA Chip test. The interpretation of HPV DNA sequencing was impossible in nine cases (3.2%). The HPV DNA sequencing test of 95 cases of HPV-chip other type showed that the sequencing types from 94 cases (95.8%) were absent types in HPV DNA Chip test. In sequencing test of HPV-chip other type, HPV-81 (20.9%), HPV-62 (14.7%), HPV-84 (13.7%), and HPV-61 (13.7%) were frequently detected.

Conclusion. HPV DNA Chip is an accurate method for detecting the 24 HPV genotypes.

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Keywords: Cervix; HPV DNA Chip; HPV DNA sequencing; HPV-chip other type

Specific cervical lesions may be predicted, thus providing appropriate patient care according to HPV types [11]. In several studies demonstrated that persistent HPV infection with a high-risk type is strongly associated with cervical dysplasia and carries a greater risk of subsequent progression to cervical cancer [12,13]. In this study, we evaluated the accuracy of HPV DNA Chip test for detection and typing of HPV in cervical lesions by comparing with result of HPV DNA sequencing of same samples. In addition, we desired to find out the types which were not detected in HPV DNA Chip test by HPV DNA sequencing of HPV-chip other types.

Materials and methods

Study subjects

HPV DNA sequencing was performed in 377 cervical samples obtained from 377 patients, in which positive reaction had been shown by HPV DNA Chip test. These samples were composed of 282 samples, in which specific HPV genotypes had been detected in HPV DNA Chip test and 95 samples, in which had shown amplified HPV-PCR product, but specific HPV genotypes had not been detected (HPV-chip other type samples). The 282 study samples included 266 samples of single HPV genotype (single infection) and 16 of multiple genotypes (multiple infection) in HPV DNA Chip test. The types of HPV DNA sequencing were compared with the types of HPV DNA Chip test. In cases of multiple infection, when the type of HPV DNA sequencing was one of the types of HPV DNA Chip test, we considered that sequencing type agrees with the type of HPV DNA Chip. In the HPV DNA sequencing test of 95 HPV-chip other type samples, we checked whether the sequencing types of HPV-chip other type samples are present or not in HPV DNA Chip test.

HPV genotyping in HPV DNA Chip test

We used the HPV DNA Chip, PCR-based DNA microarray system, as an HPV genotyping method (provided by MyGene Company, Seoul, South Korea). The HPV DNA Chip contains 24 type from high-risk types (HPV-16, HPV-18, HPV-31, HPV-33, HPV-35, HPV-39, HPV-45, HPV-51, HPV-52, HPV-53, HPV-56, HPV-58, HPV-59, HPV-66, and HPV-68) and 9 types of low-risk types (HPV-6, HPV-11, HPV-16, HPV-90, HPV-42, HPV-43, HPV-43, HPV-54, HPV-70). Twenty-four type-specific 30-mer oligonucleotide probes containing an amine group at the 5’ terminus were immobilized onto a chip slide glass. A slide has eight chambers, and each chamber is used for a test. Therefore, a slide tests eight samples at one time. Primers of HPV-genotyping was amplification, and labeled by a single dye, indocarbocyanine-DUTP (MEN Life Science Products, Inc, MA). Using consensus GP5+/GP6+/primers, β-Globin was amplified using PCR as an internal control. The PCR products of all samples were detected by electrophoresis through a 2.5% agarose gel, and the probe size of HPV DNA was 130 base pairs (bp). Ten microliter of the HPV-amplified product was desorbed for 5 min at 95°C. The samples were mixed with a hybridization solution (MyGene Co., Seoul, Korea) and then applied onto the DNA Chip. Hybridization was performed at 43°C for 90 min and was followed by washing 3×SSPE for 5 min at 5°C and 1×SSPE for 5 min and drying at room temperature.

Hybridized HPV DNA was visualized using a DNA Chip scanner (Scannertry Inc, GSI Lumonics® Ottawa, Ontario, Canada). HPV amplification was hybridized with corresponding type-specific oligonucleotide probe and visualized on HPV DNA Chip slides as double positive spots (Fig. 1) when HPV DNA was present in amplified PCR product. The samples that showed a positive band of 150 bp on the gel electrophoresis were negative on the HPV DNA Chip slide were designated as HPV-negative. None of the negative controls (without DNA) revealed HPV positivity.

HPV genotyping in HPV DNA sequencing

The primed PCR product was added to the sequencing reaction mixture. Sequencing was performed bidirectionally

Fig. 1. Examples of the HPV DNA Chip. The HPV DNA Chip is available for detection of 24 HPV genotypes, all of which are amplified from HPV-PCR and subsequently hybridized to oligonucleotide probes that are specific to each genotype. The positive signal was expressed in double spots. (A) A single infection of HPV56. (B) A single infection of HPV55. (C) A single infection of HPV551. (D) A double infection of HPV55 and 53.
Human papillomavirus (HPV) genotyping by HPV DNA chip in cervical cancer and precancerous lesions

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Objective: The human papillomavirus (HPV) is a well-known cause of cervical cancer. HPV tests are used as an adjunctive test to detect the false-negative rate of cytological screening. However, attempts are being made to replace the cytological screening with HPV tests. Therefore, this study was performed to examine the possibility of using HPV tests as screening tests.

Materials and methods: The results of the tests that were performed at the same time including the ThinPrep cytology, the high-risk group hybrid capture II (HC-II) test, the HPV DNA chip (HD-C) test, and a punch biopsy were compared in 400 women who were referred to us due to abnormal cytology or cervicogram. The accuracy of each test was then evaluated, and the type of virus was investigated using a HD-C test.

Results: The positive predictive values detected by the high-risk group HC-II test and HD-C test according to the histological diagnosis outcomes were 58.8 and 53.8%, respectively, for cervicitis; 91.3 and 91.5%, respectively, for cervical intraepithelial neoplasia 1 (CIN I); 88.1% and 81.0%, respectively, for CIN II; 86.6 and 84.2%, respectively, for CIN III, and 92.5 and 98.7%, respectively, for cancer (in 53 patients). The most prevalent types of HPV according to the HPV tests were types 16, 58, 18, and 52 in which type 16 was detected in the most advanced lesions. The sensitivity was 88.4% for the ThinPrep cytology, 89.9% for the HC-II for the high-risk group, and 86.2% for the HD-C test.

Conclusion: These results suggest the possibility of using the HC-II and HD-C tests as screening tests, which have a similar sensitivity as the ThinPrep cytology. Nonetheless, randomized controlled trials will be needed before the actual application of the HPV tests as screening tests. Despite the fact that the importance of HPV type 16 in cancer development was confirmed, the prevalence of types 58 and 52 were

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HPV chip test & HC2
Comparison of the Hybrid Capture II Assay With the Human Papillomavirus DNA Chip Test for the Detection of High-Grade Cervical Lesions

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Abstract: Cervical intraepithelial neoplasia (CIN) 2 is used as the threshold for treatment decisions. This study was conducted to evaluate the clinical efficacy of the Hybrid Capture II assay (HC2) and the human papillomavirus (HPV) DNA chip test (HDC) for detecting HPV in high-grade cervical lesions CIN2 or greater, including adenocarcinoma (CIN2+). Seven hundred forty-one women with abnormal cervical cytology were evaluated with the HC2, the HDC, and histological assessment of the cervix. The overall agreement of the 2 HPV tests was 88.8% (κ value, 0.61). Of 615 high-risk HPV-positive specimens by the HC2, 571 (92.8%) were HDC-positive. Both tests were performed similarly on CIN2+ samples; the sensitivities of the HC2 and HDC as predictors of CIN2+ were 93.4 and 92.6%, respectively. In 83 cases of discrepancies between the HC2 and HDC, genotyping of 39 HC2-negative/HDC-positive cases revealed 13 HPV-53, 8 HPV-58, 7 HPV-16, 6 HPV-18, 2 HPV-68, 1 HPV-31, 1 HPV-45, and 1 HPV-66. In 515 patients with CIN2+, HPV-16 (45.0%) was the most common type; the next most common types were HPV-58 (20.8%), HPV-18 (16.1%), HPV-31 (6.6%), and HPV-33 (6.6%). Human papillomavirus types 16, 58, and 18 were more likely associated with CIN2+ (P < 0.05). In conclusion, the HDC is a reliable diagnostic tool for the detection of CIN2+. In addition, the HDC provides useful information regarding viral genotypes.

Key Words: High-grade cervical lesions, HPV DNA chip test, Human papillomavirus, Hybrid capture II assay.
Significance of human papillomavirus genotyping with high-grade cervical intraepithelial neoplasia treated by a loop electrosurgical excision procedure

Woo Dae Kang, MD, PhD; Min Jeong Oh, MD; Seok Mo Kim, MD, PhD; Jong Hee Nam, MD, PhD; Chang Soo Park, MD, PhD; Ho Sun Choi, MD, PhD

OBJECTIVE: This study was conducted to determine whether the human papillomavirus (HPV) genotype by the HPV DNA chip test (HDC) is predictive of residual or recurrent high-grade cervical intraepithelial neoplasia (CIN) 2-3 following a loop electrosurgical excision procedure (LEEP).

STUDY DESIGN: Between January 2001–February 2007, 672 patients with CIN2-3 were treated by a LEEP and followed up with cytology, the hybrid capture II assay, and the HDC.

RESULTS: A total of 37 (5.5%) patients developed a recurrence, and those who developed a recurrence tested positive for the same high-risk (HR) HPV genotype before and after the LEEP. The same HR-HPV genotype by the HDC during the follow-up had a sensitivity and negative predictive value of 100% for detecting residual/recurrent disease. Persistent HPV-16 and HPV-18 were significantly associated with recurrent CIN2-3 ($P < .05$).

CONCLUSION: Persistent infection with the same HR-HPV genotype, especially HPV-16 and HPV-18, should be considered a risk factor for developing residual/recurrent CIN2-3.

Key words: high-grade cervical intraepithelial neoplasia, high-risk human papillomavirus testing, loop electrosurgical excision procedure

HPV-18 is a poor prognostic factor, unlike the HPV viral load, in patients with stage IB–IIA cervical cancer undergoing radical hysterectomy

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ABSTRACT

Objectives. This study was conducted to determine the prognostic significance of the human papillomavirus (HPV) genotype using the HPV DNA chip (HDC) test and the HPV viral load by the hybrid capture II assay (HC2) in FIGO stage IB–IIA cervical cancer undergoing radical hysterectomy.

Methods. Between January 2001 and December 2005, 204 consecutive patients who underwent radical hysterectomy with pelvic lymphadenectomy for International Federation of Gynecology and Obstetrics (FIGO) stage IB1–IIB cervical cancer were retrospectively reviewed. The Cox proportional hazard models adjusted for covariates were used for analyses and a receiver operating characteristic (ROC) curve was used to determine the HPV viral load in predicting disease progression.

Results. Of the 204 cases, the HDC was positive in 195 (95.6%) and the HC2 was positive in 192 (94.1%). The 5-year progression-free survival (PFS) was 78.4%. On multivariate analysis, HPV-18 positivity was an independent prognostic factor predictive for disease progression. The risk of recurrence was higher for HPV-18 positivity (hazard ratio = 2.664; 95% confidence interval [CI], 1.437–4.938; P = 0.003). The 5-year PFS rate for patients who were HPV-18-negative was 83.8%, which was higher than the 5-year PFS rate for patients who were HPV-18-positive (54.1%; P < 0.001). The area under the ROC curve for the HPV viral load was 0.550 (P = 0.314; 95% CI, 0.455–0.644).

Conclusions. The HPV-18 genotype is a reliable prognostic factor of early-stage cervical cancer; however, the HPV viral load may not be helpful in predicting disease prognosis.
Is vaccination with quadrivalent HPV vaccine after loop electrosurgical excision procedure effective in preventing recurrence in patients with high-grade cervical intraepithelial neoplasia (CIN2–3)?

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HIGHLIGHTS

• HPV vaccination after treatment significantly reduces the risk of developing recurrent CIN2–3 related to the vaccine HPV types.
• HPV vaccination after treatment may be considered in preventing recurrence of CIN2–3.

ARTICLE INFO

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High grade CIN
HPV
LEEP
Vaccine

ABSTRACT

Objectives. This study was conducted to determine whether vaccination with the quadrivalent human papillomavirus (HPV) vaccine after loop electrosurgical excision procedure (LEEP) for high-grade cervical intraepithelial neoplasia (CIN2–3) is effective in preventing recurrence of CIN2–3.

Methods. Between August 2007 and July 2010, 737 patients aged 20–45 years who were diagnosed with CIN2–3 were treated by LEEP and followed. Three hundred and sixty patients were vaccinated with the quadrivalent HPV vaccine after LEEP (vaccination group), and 377 patients were followed without vaccination (non-vaccination group). The vaccination group received the first dose at 1 week after LEEP and the remaining two doses two and six months later. Post-LEEP follow-up was performed at 3, 6, 9, 12, 18, and 24 months during the first 2 years and yearly thereafter.

Results. Irrespective of causal HPV type, 36 (4.9%) patients developed recurrence in the vaccination group (360 patients), 9 patients (2.5%) developed recurrence, whereas 27 patients (7.2%) in the non-vaccination group (377 patients) developed recurrence. In patients infected with HPV of 16 and/or 18 type, 5 patients (2.5%) in the vaccination group (197 patients) and 18 patients (8.5%) in the non-vaccination group (211 patients) developed recurrent disease related to vaccine HPV types (HPV 16 or 18 types) after LEEP ($P < 0.01$).

Multivariate analysis showed that no vaccination after LEEP was an independent risk factor for recurrent CIN2–3 ($HR = 2.840; 95\% \text{ confidence interval}, 1.335–6.042; P < 0.01$).

Conclusions. Vaccination with the quadrivalent HPV vaccine after treatment may be considered in...
HPV chip test & HPV PCR
HPV chip test & HC2

HPV genotype in CIN2-3

HPV genotype in LEEP

HPV genotype in RH

HPV genotype in vaccination and CIN2-3

HPV genotype in subsequent hysterectomy
Human Papillomavirus Test After Conization in Predicting Residual Disease in Subsequent Hysterectomy Specimens

Jeong-Yeol Park, MD, Dae-Yeon Kim, MD, PhD, Jong-Hyeok Kim, MD, PhD, Yong-Min Kim, MD, PhD, Young-Tak Kim, MD, PhD, and Joo-Hyun Nam, MD, PhD

OBJECTIVE: To estimate the effectiveness of the human papillomavirus (HPV) test performed after conization in predicting residual disease in patients who subsequently underwent hysterectomy.

METHODS: A total of 115 patients who underwent hysterectomy after conization caused by cervical intraepithelial neoplasia grade 3 (CIN 3) and microinvasive cervical cancer (IA1 cancer) were included in this prospective study. All patients underwent HPV testing with a liquid hybridization assay immediately before hysterectomy. Differences in sensitivity, specificity, and accuracy between resection margin and the HPV test in predicting residual disease in subsequent hysterectomy samples were estimated using the McNemar exact test.

RESULTS: Univariate analysis showed that age, parity, menopausal status, glandular extension, and severity of disease were not predictive for residual disease, but positive resection margin and positive HPV tests were significant factors for predicting residual disease. These factors were also significant in a multivariable analysis: positive resection margin: 85.9%, odds ratio (OR) 3.09, 95% confidence interval (CI) 1.91–8.03; P = 0.02; positive HPV test: 75.6%, OR 11.02, 95% CI 4.01–30.49, P < 0.001. With resection margin, the sensitivity, specificity, and accuracy in predicting residual disease were 73.5%, 53%, and 61%, respectively, whereas, with the HPV test, these values were 85%, 67%, and 73%, respectively (P = 0.45, 0.86, and 0.44, respectively). Of patients with positive resection margins, 79% of HPV-negative patients had no residual disease. Of patients with negative resection margins, no HPV-negative patient had residual disease.

CONCLUSION: The HPV test after conization was significantly more accurate than resection margin for predicting residual disease. The predictive value of resection margin for predicting residual disease was much improved when used in combination with the HPV test. Use of the HPV test is recommended for identifying patients for subsequent hysterectomy after conization for CIN 3 and IA1 cancer.

(Level of Evidence: III)

Obstet Gynecol 2009;114(47–92)

Introduction

In the past, the standard therapy for patients with cervical intraepithelial neoplasia (CIN) III and microinvasive carcinoma was usually hysterectomy, especially for women younger than 40 years old. In recent years, however, there has been a shift toward more conservative surgery in the treatment of gynecologic malignancy and premalignant disease, including conization for CIN III and microinvasive carcinoma. These patients have been treated by excisional techniques, including cold knife conization (CKC) and large loop excision of the transformation zone (LEEP). Hysterectomy has therefore replaced more conservative options, such as laser vaporization or loop excision of the transformation zone (LEEP) [3]. As a result, hysterectomy is now rarely performed routinely, provided that no risk factors are present and the absence of residual disease after conization can be accurately predicted [3–5].

Among the demographic and clinicopathologic factors that may predict residual disease after conization are age, menopausal status, parity, severity of disease, postcone endocervical curettage, surgical margin, endocervical gland involvement, and positive resection margin. The authors did not report any potential conflicts of interest.

Cervical cancer is one of the most common cancers in women worldwide, and its incidence is increasing. To reduce the incidence of cervical cancer, it is important to identify patients who are at high risk for cervical cancer and to develop effective screening and treatment strategies. Therefore, a reliable test for predicting residual disease after conization is important for the conservative treatment and counseling of patients with CIN 3 and IA1 cancer, both for the patient and the physician. Although several demographic and clinicopathologic factors, including age, parity, menopausal status, severity of lesion, glandular extension, and resection margin, have been reported to be predictive for residual disease after conization, resection margin remains the gold-standard technique for prediction of residual disease after conization. However, residual disease can be found subsequently in up to 2–31% of patients with negative resection margins.

This may be due to multiple lesions that were not resected during conization, by contrast, residual disease is not found in up to 10–60% of patients with positive resection margins. This may be because residual
A human papillomavirus (HPV)-16 or HPV-18 genotype is a reliable predictor of residual disease in a subsequent hysterectomy following a loop electrosurgical excision procedure for cervical intraepithelial neoplasia 3

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ABSTRACT

Objective: This study was conducted using the human papillomavirus (HPV) DNA chip test (HDC), in order to determine whether the HPV genotype is a predictor of residual disease in a subsequent hysterectomy following a loop electrosurgical excision procedure (LEEP) for cervical intraepithelial neoplasia (CIN) 3.

Methods: Between January 2002 and February 2015, a total of 189 patients who underwent a hysterectomy within 6 months of LEEP caused by CIN 3 were included in this study. We analyzed their epidemiological data, pathological parameters, high-risk HPV (HR-HPV) load as measured by the hybrid capture II assay, and HR-HPV genotype as measured by the HDC. A logistic regression model was used to analyze the relationship between covariates and the probability of residual disease in subsequent hysterectomy specimens.

Results: Of the 189 patients, 92 (48.7%) had residual disease in the hysterectomy specimen, CIN 2 in seven patients, CIN 3 in 79 patients, IAI cancer in five patients, and IAI2 cancer in one patient. Using multivariate analysis, the results were as follows: cone margin positivity (odds ratio [OR], 2.43; 95% CI, 1.18 to 5.29; p < 0.05), HPV viral load ≥ 220 relative light unit (OR, 2.98; 95% CI, 1.38 to 6.43; p < 0.01), positive endocervical cytology (OR, 8.97; 95% CI, 3.81 to 21.13; p < 0.001), and HPV-16 or HPV-18 positivity (OR, 9.07; 95% CI, 3.86 to 21.30; p < 0.001).

Conclusion: The HPV-16 or HPV-18 genotype is a reliable predictive factor of residual disease in a subsequent hysterectomy following a LEEP for CIN 3.

Keywords: Cervical Intraepithelial Neoplasia; Conization; Human Papillomavirus;
HPV chip test & HPV PCR
  HPV chip test & HC2
  HPV genotype in CIN2-3
  HPV genotype in LEEP
  HPV genotype in RH
  HPV genotype in vaccination and CIN2-3
  HPV genotype in subsequent hysterectomy
  ?
Cervical stenosis

- Cervical stenosis and inadequate colposcopy after LEEP
  - prevent a correct follow-up with the risk of unseen relapses
Management of Women with Biopsy-confirmed Cervical Intraepithelial Neoplasia — Grade 2 and 3 (CIN2,3)*

*Management options will vary in special circumstances or if the woman is pregnant or ages 21-24

If CIN2,3 is identified at the margins of an excisional procedure or post-procedure ECC, cytology and ECC at 4-6mo is preferred, but repeat excision is acceptable and hysterectomy is acceptable if re-excision is not feasible.

Adequate Colposcopy

Either Excision\(^1\) or Ablation of T-zone\(^*\)

2x Negative Results

Cotesting at 12 and 24 months

Inadequate Colposcopy or Recurrent CIN2,3 or Endocervical sampling is CIN2,3

Diagnostic Excisional Procedure\(^*\)

Any test abnormal

Colposcopy With endocervical sampling

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HPV genotype and recurrence after LEEP for CIN2-3

ONCOLOGY

Significance of human papillomavirus genotyping with high-grade cervical intraepithelial neoplasia treated by a loop electrosurgical excision procedure

Woo Dae Kang, MD, PhD; Min Jeong Oh, MD; Seok Mo Kim, MD, PhD; Jong Hee Nam, MD, PhD; Chang Soo Park, MD, PhD; Ho Sun Choi, MD, PhD

OBJECTIVE: This study was conducted to determine whether the human papillomavirus (HPV) genotype by the HPV DNA chip test (HDC) is predictive of residual or recurrent high-grade cervical intraepithelial neoplasia (CIN) 2–3 following a loop electrosurgical excision procedure (LEEP).

STUDY DESIGN: Between January 2001–February 2007, 672 patients with CIN2–3 were treated by a LEEP and followed up with cytology, the hybrid capture II assay, and the HDC.

RESULTS: A total of 37 (5.5%) patients developed a recurrence, and those who developed a recurrence tested positive for the same high-risk (HR) HPV genotype before and after the LEEP. The same HR-HPV genotype by the HDC during the follow-up had a sensitivity and negative predictive value of 100% for detecting residual/recurrent disease. Persistent HPV-16 and HPV-18 were significantly associated with recurrent CIN2–3 (P < .05).

CONCLUSION: Persistent infection with the same HR-HPV genotype, especially HPV-16 and HPV-18, should be considered a risk factor for developing residual/recurrent CIN2–3.

Key words: high-grade cervical intraepithelial neoplasia, high-risk human papillomavirus testing, loop electrosurgical excision procedure

Introduction

• 이미 알려진 사실을 기술
• 아직 알려져 있지 않은 사실이나 논쟁의 여지가 있는 사항들을 기술
• 위의 과정을 통해 연구가 추구하는 목적이 필요성을 유도
• 연구 목적을 분명히 한다.
• 과도한 문헌고찰을 하지 않는다.
• 되도록 간략하게 쓴다.

노주원 선생님. 동국대학교. 2015 JGO workshop
High-grade cervical intraepithelial neoplasia (CIN; CIN2-3), if left untreated, bears a significant risk of developing into invasive carcinoma. Persistent high-risk human papillomavirus (HR-HPV) infections are more strongly associated with the development of CIN2-3 and are considered the first step in the progression to cervical carcinoma. When CIN2-3 is detected during fertile age, conservative treatment with a loop electrosurgical excision procedure (LEEP) is mandatory for eradicating CIN2-3 and for preserving reproductive function. Correct management of the same lesions diagnosed after menopause is still being debated. The incidence of residual/recurrent disease after LEEP varies between 5% and 30%, and follow-up and retreatment are needed once lesions have been identified.

Regardless of women’s age, satisfactory follow-up is the main requirement for the conservative management of CIN2-3. In the follow-up of women after LEEP for CIN2-3, the main objective is early detection of recurrent disease because of the risk of progression to invasive carcinoma if effective treatment is not rendered. HR-HPV detection techniques have recently been proposed during follow-up of women treated for CIN2-3 because of their high sensitivity and negative predictive value in detecting recurrent disease—a good index of disease clearance. Thus, growing evidence indicates that HR-HPV, in conjunction with cytology, should be useful for monitoring women treated for CIN2-3.

Cervical stenosis and inadequate colposcopy after LEEP, especially in postmenopausal women, are the main factors preventing correct follow-up, with a risk of unseen relapses. Some studies described a higher risk of cervical stenosis after LEEP in postmenopausal women. Squamocolumnar junction, which is usually exocervical in fertile age, often becomes endocervical after menopause.

The aim of this study was to determine, using the HPV DNA Chip (HDC) test (MyGene Co, Seoul, South Korea), whether the human papillomavirus (HPV) genotype is predictive of recurrent disease during follow-up of CIN2-3 after LEEP in postmenopausal women.
Method

- 명확한 연구 대상 (disease, number)
- Inclusion criteria
- Exclusion criteria
- 관찰 항목과 관찰 방법
- 용어의 정의
- 검사 방법
- 자료평가를 위한 통계학적 분석법
- IRB 기술

노주원 선생님. 동국대학교. 2015 JGO workshop
METHODS

We retrospectively reviewed the records of all postmenopausal women with histologically confirmed CIN2-3 who had been treated by LEEP at the Department of Obstetrics and Gynecology of Chonnam National University Hospital between January 2007 and February 2013.

Two hundred six postmenopausal women were considered eligible for the study after they fulfilled the following criteria: (a) histologically confirmed CIN2-3 by LEEP; (b) availability of both pre-LEEP and post-LEEP HR-HPV test results from the HDC test (MyGene Co) and the Hybrid Capture II (HC2) assay (Digene Co, Gaithersburg, MD); (c) had not received HPV quadrivalent vaccine or bivalent vaccine before the diagnosis of CIN2-3; (d) at least 12 months of spontaneous amenorrhea at LEEP; and (e) followed for a minimum of 2 years. We excluded women who were diagnosed with residual CIN2-3 or who underwent hysterectomy within 6 months of LEEP. Epidemiologic data, HR-HPV test data from HDC and HC2, and pathology data were obtained from medical records.

LEEP was performed under local anesthesia using wire loop electrodes with a diathermy apparatus set. A section was placed at 12 o’clock position in LEEP specimens for orientation, and the specimens were fixed in 10% formalin for pathologic examination.

Women underwent postoperative examination at 3, 6, 9, 12, 18, and 24 months during the first 2 years and yearly thereafter. At every visit after LEEP, HPV DNA tests (HC2 and HDC) and cytology were performed in all women, and colposcopy/endocervical sampling was carried out if the HPV DNA test yielded positive results or if repeated cytology revealed atypical squamous cells of undetermined significance or greater. If CIN2-3 was reported histologically at the margins of an excised specimen or in an endocervical sample obtained immediately after LEEP, endocervical sampling was additionally performed at 3 and 6 months. Colposcopy-directed punch biopsies of the cervix were taken in the case of any suspected area after application of 5% acetic acid. When the lesion was not visible or was only partially visible, additional endocervical curettage was performed.

Criteria for residual/recurrent disease were based on positive histologic results for colposcopy-directed biopsy or endocervical curettage. Women with histologically confirmed CIN2-3 at 3-month follow-up after treatment were considered to have residual disease. Women diagnosed with CIN2-3 on biopsies on the next follow-up (from 6 mo onward) were considered to have recurrent disease. For statistical analysis, the results of cervical biopsies obtained during follow-up were grouped as negative in the presence of normal findings/cervicitis or CIN1 and as positive in the presence of CIN2 or CIN3. Positive histologic results during follow-up were considered recurrent disease. The study protocol was evaluated and approved by the Institutional Review Board at Chonnam National University Hospital.
HC2 assay
The sample was collected by placing a cytobrush into the exocervix and by rotating the brush three times; the sample was kept frozen at -20°C in a collection tube (Digene Co) until needed. Denatured single-strand DNA was hybridized with a RNA researcher of the mixed HR-HPV group. This reaction mixture was placed in a microtiter well coated with antibodies to the RNA/DNA hybrid. After RNA/DNA hybrid–antibody bonding, the mixture was reacted with alkaline phosphatase–conjugated antibodies and washed, and lum-PHospho 530 was added for reaction with the dioxetane-based chemiluminescent substrate. Alkaline phosphatase was added to obtain luminescent light, which was measured with a luminometer and expressed in relative light units. The solution containing HPV-16 DNA 1 pg/mL was used as a positive control group for the HR-HPV group. Relative light units for all samples were set to relative brightness in comparison with the positive control group. This ratio was considered positive if 1.0 or greater and considered negative if 1.0 or less. The samples were analyzed for the presence of 13 types of HR-HPV groups (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68).

HDC test
We used HDC, a polymerase chain reaction (PCR)-based DNA microarray system, as an HPV genotyping method for HPV typing. HDC contains 24 type-specific probes; 15 probes are high-risk types (16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, and 68) and 9 probes are low-risk types (6, 11, 34, 40, 42, 43, 44, 54, and 70). Briefly, DNA was isolated from a swab sample using a DNA isolation kit (MyGene Co), and target L1 regions of HPV DNA were amplified and labeled by a single dye (indocarbocyanine-dUTP; NEN Life Science Products Inc, Boston, MA). PCR products of all samples were detected by electrophoresis with a 2.5% agarose gel. The samples were mixed with a hybridization solution (MyGene Co). Hybridization was performed at 43°C for 90 minutes. Hybridized HPV DNA was visualized using a DNA chip scanner (Scanarray Lite; GSI Lumronics, Ottawa, Ontario, Canada). Fifteen types of HR-HPV positivity were used to assess HDC performance.

Statistical analysis
Relationships between recurrence with post-LEEP HPV status and other clinical factors were determined by Student’s t test or Fisher’s exact test. All P values reported are two-sided, and P < 0.05 was considered statistically significant. Agreement between tests was assessed by Cohen’s κ statistic, and P value was calculated using McNemar’s test (between 0.00 and 0.20, poor agreement; between 0.21 and 0.40, fair agreement; between 0.41 and 0.60, moderate agreement; between 0.61 and 0.80, substantial agreement; between 0.81 and 1.00, near-perfect agreement). A receiver operating characteristic (ROC) curve was used to determine the clinically most useful cutoff value of HR-HPV viral load for predicting recurrent disease. Data were analyzed using the Statistical Package Service Solution software (SPSS for Windows, standard version 21.0; SPSS Inc, Chicago, IL). Ninety-five percent CIs were calculated.

자료평가를 위한 통계학적 분석
검사 방법
# Table 1. Participant characteristics

<table>
<thead>
<tr>
<th></th>
<th>No recurrence (n = 180)</th>
<th>Recurrence (n = 26)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean (SD)</td>
<td>57.2 (3.6) [53-76]</td>
<td>57.3 (4.7) [52-75]</td>
<td>0.5</td>
</tr>
<tr>
<td>Initial cytology</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ASCUS</td>
<td>22 (12)</td>
<td>3 (11)</td>
<td>0.9</td>
</tr>
<tr>
<td>LSIL</td>
<td>12 (7)</td>
<td>1 (4)</td>
<td>0.9</td>
</tr>
<tr>
<td>HSIL</td>
<td>146 (81)</td>
<td>22 (85)</td>
<td>0.9</td>
</tr>
<tr>
<td>CIN at LEEP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CIN2</td>
<td>35 (19)</td>
<td>5 (19)</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>CIN3</td>
<td>145 (81)</td>
<td>21 (81)</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>Pre-LEEP HC2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>5 (3)</td>
<td>1 (4)*</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>Positive</td>
<td>174 (97)</td>
<td>25 (96)</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>Pre-LEEP HDC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>5 (3)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>175 (97)</td>
<td>26 (100)</td>
<td></td>
</tr>
<tr>
<td>Cone margin involvement</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>169 (97)</td>
<td>15 (58)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Positive</td>
<td>11 (6)</td>
<td>11 (42)</td>
<td></td>
</tr>
<tr>
<td>Endocervical cytology</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>168 (93)</td>
<td>20 (77)</td>
<td>0.02</td>
</tr>
<tr>
<td>Positive</td>
<td>12 (7)</td>
<td>6 (23)</td>
<td></td>
</tr>
<tr>
<td>Follow-up cytology</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>165 (92)</td>
<td>9 (35)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Positive</td>
<td>15 (8)</td>
<td>17 (65)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>ASC or greater</td>
<td>163 (91)</td>
<td>14 (48)*</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Positive</td>
<td>17 (9)</td>
<td>25 (96)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Post-LEEP HC2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>162 (90)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>18 (10)</td>
<td>26 (100)</td>
<td></td>
</tr>
</tbody>
</table>

Data are presented as n (%), unless otherwise stated.

ASCUS, atypical squamous cells of undetermined significance; LSIL, low squamous intraepithelial lesion; HSIL, high squamous intraepithelial lesion; CIN, cervical intraepithelial neoplasia; LEEP, loop electrosurgical excision procedure; HC2, Hybrid Capture II; HDC, HPV DNA Chip.

# Table 2. Level of concordance between high-risk human papillomavirus tests

<table>
<thead>
<tr>
<th>Specimens with HDC</th>
<th>Negative</th>
<th>Positive</th>
<th>Total</th>
<th>Specimens</th>
</tr>
</thead>
<tbody>
<tr>
<td>HC2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>5</td>
<td>2</td>
<td>7</td>
<td>(3.4)</td>
</tr>
<tr>
<td>Positive</td>
<td>0</td>
<td>199</td>
<td>199</td>
<td>(96.6)</td>
</tr>
<tr>
<td>Total</td>
<td>5 (2.4)</td>
<td>201 (97.6)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are presented as n (%).

HC2, Hybrid Capture II; HDC, HPV DNA Chip.
*Agreement (absolute agreement, 99.0%) between tests was assessed by Cohen’s κ statistic (0.828). P value (<0.001) was calculated using McNemar’s test.

# Table 3. Comparison of groups, by high-risk human papillomavirus genotype infection after LEEP

<table>
<thead>
<tr>
<th>Persistent high-risk human papillomavirus infection</th>
<th>Different subtype (n = 10)</th>
<th>Same subtype (n = 34)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial cytology</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ASCUS</td>
<td>4 (40)</td>
<td>6 (18)</td>
<td>0.3</td>
</tr>
<tr>
<td>LSIL</td>
<td>1 (10)</td>
<td>2 (6)</td>
<td></td>
</tr>
<tr>
<td>HSIL</td>
<td>5 (50)</td>
<td>26 (76)</td>
<td></td>
</tr>
<tr>
<td>CIN at LEEP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CIN2</td>
<td>3 (30)</td>
<td>6 (18)</td>
<td>0.7</td>
</tr>
<tr>
<td>CIN3</td>
<td>7 (70)</td>
<td>28 (82)</td>
<td></td>
</tr>
<tr>
<td>Cone margin involvement</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>1 (10)</td>
<td>12 (35)</td>
<td>0.3</td>
</tr>
<tr>
<td>Positive</td>
<td>0 (0)</td>
<td>6 (18)</td>
<td>0.4</td>
</tr>
<tr>
<td>Positive endocervical cytology at LEEP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Recurrence</td>
<td>0 (0)</td>
<td>26 (77)</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Data are presented as n (%).

LEEP, loop electrosurgical excision procedure; ASCUS, atypical squamous cells of undetermined significance; LSIL, low squamous intraepithelial lesion; HSIL, high squamous intraepithelial lesion; CIN, cervical intraepithelial neoplasia.

# Table 4. Sensitivity, specificity, and predictive values of different tests in predicting recurrent CIN2-3

<table>
<thead>
<tr>
<th>Endocervical cytology</th>
<th>Sensitivity n (%)</th>
<th>Specificity n (%)</th>
<th>PPV n (%)</th>
<th>NPV n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>6/26 (23.1)</td>
<td>198/198 (99.5)</td>
<td>11/11 (100)</td>
<td>58/58 (100)</td>
</tr>
<tr>
<td>Positive</td>
<td>14/14 (100)</td>
<td>177/180 (98.4)</td>
<td>7/7 (100)</td>
<td>171/171 (100)</td>
</tr>
</tbody>
</table>

Data are presented as n (%).

LEEP, loop electrosurgical excision procedure; HC2, Hybrid Capture II; HDC, HPV DNA Chip.

# Table 5. Correlations between pre-LEEP high-risk human papillomavirus genotypes, by HDC and recurrent disease

<table>
<thead>
<tr>
<th>Persistent high-risk human papillomavirus infection</th>
<th>Different subtype (n = 10)</th>
<th>Same subtype (n = 34)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial cytology</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ASCUS</td>
<td>4 (40)</td>
<td>6 (18)</td>
<td>0.3</td>
</tr>
<tr>
<td>LSIL</td>
<td>1 (10)</td>
<td>2 (6)</td>
<td></td>
</tr>
<tr>
<td>HSIL</td>
<td>5 (50)</td>
<td>26 (76)</td>
<td></td>
</tr>
<tr>
<td>CIN at LEEP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CIN2</td>
<td>3 (30)</td>
<td>6 (18)</td>
<td>0.7</td>
</tr>
<tr>
<td>CIN3</td>
<td>7 (70)</td>
<td>28 (82)</td>
<td></td>
</tr>
<tr>
<td>Cone margin involvement</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>1 (10)</td>
<td>12 (35)</td>
<td>0.3</td>
</tr>
<tr>
<td>Positive</td>
<td>0 (0)</td>
<td>6 (18)</td>
<td>0.4</td>
</tr>
<tr>
<td>Positive endocervical cytology at LEEP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Recurrence</td>
<td>0 (0)</td>
<td>26 (77)</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Data are presented as n (%).

LEEP, loop electrosurgical excision procedure; ASCUS, atypical squamous cells of undetermined significance; LSIL, low squamous intraepithelial lesion; HSIL, high squamous intraepithelial lesion; CIN, cervical intraepithelial neoplasia.

*Significantly higher than the results for other high-risk human papillomavirus genotype infections (χ² test; P < 0.05).

*High-risk human papillomavirus types 33, 35, 39, 45, 51, 59, 66, and 68.
Discussion

- Start: State the research objective stated in the introduction. Respond to the questions raised in the introduction.
- Result: Interpret the results mentioned in the results section.
  - Compare with existing research.
  - How do other people's research results align with my findings?
  - Does my research support past results?
  - Interpret the content of graphs and tables (think about the results and graphs and tables you read).
- Importance of the research
- Limitations of the research
- Summary and conclusion

No Ju Won, Sunggum, Dongguk University, 2015 JGO workshop
DISCUSSION

Conservative treatment with LEEP is both a diagnostic procedure and a therapeutic procedure that can effectively eradicate CIN2-3 at every stage of a woman’s life.\textsuperscript{8} Treatment failure is an important concern after conservative treatment of CIN2-3. In the present study, 26 of 206 postmenopausal women (12.6\%) developed recurrent disease, a finding consistent with previously reported data,\textsuperscript{5-7} whereas the mean age of all 377 women was 36.7 years (range, 20-25 y), and 27 of these women (7.2\%) developed recurrent disease in our previous study.\textsuperscript{11} Postmenopausal women in the present study had a higher recurrence rate than younger women in the previous study. Evaluation of the menopausal cervix is difficult, given the tendency for the transition zone to recede into the cervical canal in the absence of estrogen stimulation. This makes adequate cytologic sampling challenging and adequate colposcopy evaluation often difficult. Caution should be practiced when treating postmenopausal women in the hypoestrogenic state.

Postmenopausal women should be examined by an experienced and skilled colposcopist because the most important factor determining the quality of clearance results is the accuracy of the diagnosis. The skilled colposcopist must have intimate knowledge of cytology and histopathology so that an accurate selection of women can be made. According to the American Society for Colposcopy and Cervical Pathology Consensus Guidelines,\textsuperscript{8} postconization management of women with CIN2-3 is cotesting with cytology and HPV test at 12 and 24 months. If both cotest results are negative, retesting in 3 years is recommended.

Based on meta-analyses, the HPV test detects recurrent disease earlier with a higher sensitivity compared with histologic assessment of the cut margin.\textsuperscript{12} In the present study, women with persistent HR-HPV infection detected by HDC after LEEP had a 59.16\% (26 of 44) risk of recurrence, whereas none of the women with a negative HR-HPV result by HDC had recurrence. Persistent infection with the same HR-HPV genotype was present in 16.5\% (34 of 206) of women, and it was a highly significant risk factor of recurrence (26 of 34; 76.5\%). The same HR-HPV genotype by HDC during follow-up showed a sensitivity and negative predictive value of 100\% in detecting recurrent disease.

Among 26 women with recurrent disease, 24 women had infection with a single HR-HPV genotype, but only 2 women (HPV-16/18 and HPV-16/58) with multiple HR-HPV genotype infections developed recurrence. Furthermore, HPV-18 was significantly associated with recurrence of CIN2-3 ($P < 0.05$). Persistent infection with the same HR-HPV genotype by HDC detected recurrent disease more quickly. Further diagnostic evaluation for secondary treatment and close follow-up are needed for women with persistent infection with the same HR-HPV genotype after treatment.
Table 2-5 언급한 결과에 대한 해석
이 연구의 중요성
연구의 한계점

Although pathologic margin status is generally considered as a risk factor for the development of recurrent or persistent CIN, a free margin does not always indicate complete excision because of the possibility of multifocal lesions or inadequate specimen tissue caused by ablative conization. Most women with involved margins will not develop recurrent or persistent CIN, and up to 40% of all women undergoing loop excision have pathologic margin involvement. In the current study, section margin status and endocervical cytology at the time of LEEP were positive in 24 (11.6%) and 18 (8.7%) of 206 women, but only 11 (45.8%) and 6 (33.3%) women had recurrence, respectively.

HDC is a newly developed biotechnology that may be applied for the detection and typing of HPV. The accuracy of HDC in detecting and typing HPV in cervical lesions, by comparing the results of HPV DNA sequencing using the same samples, was 257 of 282 cases (91.1%). In the current study, the degree of concordance between HDC and HC2 was 99.0% (Cohen’s κ = 0.828; near-perfect agreement), and the HPV detection rate determined by HDC was comparable to that determined by HC2. In our previous study assessing the concordance between both HPV tests in 672 women with CIN2-3, the overall agreement between the two tests was 97.3% (κ = 0.815; near-perfect agreement). The HPV detection rate determined by HDC was comparable to that determined by HC2 in women with CIN2-3.

Previous studies have reported about the association of a high HC2 viral load with the risk for recurrent CIN2-3, whereas we concluded that HR-HPV viral loads measured by HC2 were not associated with recurrent CIN2-3. In the current study, the AUC for the HR-HPV viral load measured by HC2 predicting recurrent CIN2-3 was 0.567 (P = 0.3). The HR-HPV load using variable cutoff values before LEEP was not associated with the risk of recurrent CIN2-3. We have several possible explanations for the discordant findings. First, HC2 viral load does not evaluate cell number, which can vary substantially from one sample to another. Second, global high viral load may be misleading depending on the detection of a single HR-HPV genotype or multiple HR-HPV genotypes among the 13 high-risk types.

To our knowledge, we are the first authors to show that HR-HPV genotype is predictive of recurrent CIN2-3 after LEEP in postmenopausal women. The most significant finding of this study is that persistent infection with the same HR-HPV genotype, especially HPV-18, is significantly associated with recurrence. Evaluation of the menopausal cervix treated for CIN2-3 is difficult; therefore, it is extremely important to detect the persistent types of HR-HPV genotype during follow-up in postmenopausal women to identify those at risk for developing cervical abnormalities.

Limitations of our study include the retrospective nature of the design and the relatively small sample size. In addition, HDC has not been approved by the Food and Drug Administration for verifying HR-HPV genotype. Although we noted the superiority of HPV genotyping to cytology or margin status, no direct comparison of these measures was made.
CONCLUSIONS

In postmenopausal women, persistence of the same HR-HPV genotype is a reliable prognostic marker of the recurrence of CIN2-3 after LEEP, and follow-up using cytology and HR-HPV genotype may be acceptable. This finding requires substantiation in a further large-scale prospective investigation using standardized PCR techniques for HPV detection; however, persistent infection with the same HR-HPV genotype after LEEP, especially HPV-18, should be considered a risk factor for developing recurrent CIN2-3. Such women warrant special attention with intense follow-up.

요약과 주장
Human papillomavirus genotyping as a reliable prognostic marker of recurrence after loop electrosurgical excision procedure for high-grade cervical intraepithelial neoplasia (CIN2-3) especially in postmenopausal women

Woo Dae Kang, MD, PhD, and Seok Mo Kim, MD, PhD

Abstract

Objective: This study was conducted to determine, using the HPV DNA Chip (HDC) test, whether the human papillomavirus (HPV) genotype is predictive of recurrent high-grade cervical intraepithelial neoplasia (CIN; CIN2-3) after a loop electrosurgical excision procedure (LEEP) in postmenopausal women.

Methods: Between January 2007 and February 2013, 206 postmenopausal women with CIN2-3 were treated with LEEP, followed by cytology, Hybrid Capture II (HC2) assay, and HDC test. Post-LEEP follow-up was performed at 3, 6, 9, 12, 18, and 24 months during the first 2 years and yearly thereafter.

Results: Among 206 women, HC2 yielded positive results in 199 women (96.6%) and HDC yielded positive results in 201 women (97.6%) before LEEP. The overall agreement between HDC and HC2 was 99.0%. The area under the receiver operating characteristic curve for high-risk HPV (HR-HPV) viral load measured by HC2 predicting recurrent CIN2-3 was 0.567 (P = 0.335). Twenty-six women (12.6%) developed recurrence, and those who developed recurrence tested positive for the same HR-HPV genotype before and after LEEP. The same HR-HPV genotype by HDC during follow-up had a sensitivity and negative predictive value of 100% in detecting recurrent disease. HPV-18 was significantly associated with recurrent CIN2-3 (P < 0.05).

Conclusions: Among postmenopausal women, persistent infection with the same HR-HPV genotype, especially HPV-18, should be considered a risk factor for developing recurrent CIN2-3. After LEEP, such women warrant special attention with intense follow-up.

Key Words: High-grade cervical intraepithelial neoplasia – High-risk human papillomavirus testing – Loop electrosurgical excision procedure – Menopause.
Thank you for your attentions !!