Gynecologic Oncology 130 (2013) 264–268

Contents lists available at SciVerse ScienceDirect

Gynecologic Oncology

journal homepage: www.elsevier.com/locate/ygyno

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

Article history:
Received 12 February 2013
Available online 26 April 2013

Keywords:
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% confidence interval, 1.335–6.042; $P < 0.01$).

Conclusions. Vaccination with the quadrivalent HPV vaccine after treatment may be considered in preventing recurrence of CIN2–3.

Introduction

High-grade cervical intraepithelial neoplasia (CIN2–3) bears a risk of developing invasive carcinoma if left untreated [1]. 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 [2]. Conservative treatment with a loop electrosurgical excision procedure (LEEP) is both a diagnostic and therapeutic procedure that can effectively eradicate CIN2–3 [3,4]. However, residual/recurrent disease after a LEEP varies between 5% and 30%, requiring follow-up and retreatment once lesions have been identified [5–7].

The currently available human papillomavirus (HPV) vaccines based on recombinant virus-like particles are designed to prevent HPV associated disease. Both the quadrivalent vaccine (against HPV types 6, 11, 16, and 18) and the bivalent vaccine (against types 16 and 18) are highly effective in preventing CIN2–3 or adenocarcinoma in situ in women aged 16–26 years, who are not infected with the relevant HPV type

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0090-8258/$ – see front matter
http://dx.doi.org/10.1016/j.ygyno.2013.04.050

before vaccination [8–10]. In addition, recently published data support the same efficacy of the quadrivalent HPV vaccine in susceptible women aged 24–45 years [11].

In a recent study, previous vaccination with the quadrivalent HPV vaccine among women who had surgical treatment for HPV related disease significantly reduced the incidence of subsequent HPV related disease, including high grade disease [12]. However, no studies to date have looked at the impact of HPV vaccination in preventing subsequent disease after treatment of CIN2–3.

This study was conducted to determine whether vaccination with the quadrivalent HPV vaccine among patients aged 20–45 years after LEEP for CIN2–3 is effective in preventing recurrent disease.

Methods

We retrospectively reviewed the records of all 748 patients aged 20–45 years with histologically-confirmed CIN2–3 who had been treated by LEEP at the Department of Obstetrics and Gynecology of Chonnam National University Hospital (CNUH) between August 2007 and July 2010. Seven hundred thirty-seven patients were considered eligible for the study if they fulfilled the following criteria: (a) histologically-confirmed CIN2–3 by LEEP, (b) patients in whom both pre- and post-LEEP HR-HPV test results from the HPV DNA chip test (HDC; MyGene Co., Seoul, South Korea) and the hybrid capture II assay (HC2; Digene Co., Gaithersburg, MD, USA), (c) patients who have not received the HPV quadrivalent vaccine or the bivalent vaccine before diagnosis with CIN2–3, and (d) patients who were followed for a minimum of 2 years. We excluded 5 patients who underwent hysterectomy after LEEP and 6 patients with residual CIN2–3.

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

We discussed with patients about the rationale of post-treatment vaccination after LEEP. Some patients agreed and others disagreed with vaccination after LEEP. Three hundred and sixty patients received quadrivalent HPV vaccination (vaccination group) and 377 patients did not receive quadrivalent HPV vaccination (non-vaccination group) after LEEP for CIN2–3. Patients who agreed with the quadrivalent HPV (types 6, 11, 16, and 18) L1 virus-like particle vaccine (Gardasil, Merck, Whitehouse Station, NJ, USA) received the first dose at 1 week after LEEP and the remaining two doses 2 and 6 months later. The patients underwent post-operative examination at 3, 6, 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 on all patients, and colposcopy/endocervical sampling was carried out if the HPV DNA test was positive or if repeat 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 performed at 3 and 6 months. Colposcopic 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 only partially visible, additional endocervical curettage was performed.

Criteria for residual or recurrent disease were based on positive histology of colposcopy-directed biopsy or endocervical curettage. Patients with histologically-confirmed CIN2–3 at 3 month follow-up after treatment were considered as having residual disease. Patients diagnosed with CIN2–3 on biopsies at the next follow-up (from 6 months onward) were considered as having recurrent disease. For statistical analysis, the results of cervical biopsies obtained during follow-up were grouped as negative in the presence of normal/cervicitis or CIN1 and positive in the presence of CIN2 or CIN3. Positive histologic results during follow-up were considered as recurrent disease. The study protocol was evaluated and approved by the Institutional Review Board at CNUH.

Hybrid capture II assay

The sample was collected by placing a cytobrush into the exocervix and rotating the brush 3 times; the sample was kept frozen at −20 °C in a collection tube (Digene Co) until needed. The denatured single-strand DNA was hybridized with a RNA researcher of a mixed HR-HPV group. This reaction mixture was placed in a microtiter well coated with antibodies for the RNA/DNA hybrid. After RNA/DNA hybrid-antibody bonding, the mixture was reacted with alkaline phosphatase-conjugated antibodies, washed, and lumi-Phospho 530 was added to react 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 unit. The solution containing 1 pg/mL of HPV-16 DNA was used as a positive control group for the HR-HPV group. The relative light unit for all the samples was set to the degree of relative brightness in comparison with the positive control group. This ratio was considered positive when it was 1.0 or greater and negative when it was 1.0 or less. The samples were analyzed for the presence of 13 types of the HR-HPV group (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68).

HPV DNA chip test

We used the HD-C, a PCR-based DNA microarray system as a HPV genotyping method for HPV typing. The HD-C contains 24 type-specific probes; 15 probes are HR types (16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, and 68) and 9 probes are LR 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 then target L1 regions of HPV DNA were amplified and labeled by a single dye (indocarbocyanine-dUTP; NEN Life Science Products, Inc., Boston, MA, USA). The 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 min. The hybridized HPV DNA was visualized using a DNA chip scanner (Scanarray Lite; GSI Lumonics®, Ottawa, Ontario, Canada). Fifteen types of HR-HPV positivity were used to assess HDC performance.

Statistical analysis

Statistical comparison was carried out using Student's *t*-test or Fisher exact test. Agreement between tests was assessed by Cohen's kappa statistic and *P* values were calculated using McNemar's test, with values between 0.00 and 0.20 indicating poor agreement, values between 0.21 and 0.40 indicating fair agreement, values between 0.41 and 0.60 indicating moderate agreement, values between 0.61 and 0.80 indicating substantial agreement, and values between 0.81 and 1.00 indicating near-perfect agreement. Variables showing a significant association with survival were included in multivariate analysis based on the Cox proportional-hazard model. Step-wise regression techniques were used to build multivariate models using a significance level of 0.10 to remain in the model. Statistical analyses were performed using SPSS software (version 19.0; SPSS, Inc., Chicago, IL, USA). Ninety-five percent confidence intervals were calculated. All reported *P* values are two-sided, and *P* values <0.05 were considered statistically significant.

Results

Epidemiologic data, HR-HPV test data from the HDC and HC2, and pathology data were obtained from the patients' medical records. The mean age of all 737 patients was 36.7 years. Of these, 36 patients (4.9%) were aged 20–26 years, 255 patients (34.6%) aged 27–35 years

Table 1
Patient characteristics.

	Vaccination group N = 360	Non-vaccination group N = 377	P
Age (years)			0.935
Mean ± SD	36.70 ± 6.00	36.67 ± 5.66	
Range	21–45	20–45	
Initial cytology			0.301
ASCUS	60	48	
LSIL	32	38	
HSIL	268	291	
CIN at LEEP			0.198
CIN2	54	71	
CIN3	306	306	
Cone margin			0.578
Negative	297	304	
Positive	63	73	
Endocervical cytology			0.711
Negative	344	357	
Positive	16	20	
Baseline HPV 16 or 18 by HDC			0.790
Negative	163	166	
Positive	197	211	

SD, standard deviation; 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; HPV, human papillomavirus; HDC, HPV DNA chip test.

Table 3
HR-HPV genotypes by HDC before LEEP.

	Vaccination group N = 360	Non-vaccination group N = 377	Total N = 737
None (N = 15)	7/15	8/15	15
Vaccine HR-HPV genotype (N = 408)			
16	159/197	173/211	332/408
18	28/197	26/211	54/408
16 + 18	10/197	12/211	22/408
Non-vaccine HR-HPV genotype (N = 314)			
31	19/156	18/158	37/314
33	13/156	16/158	29/314
35	6/156	8/158	14/314
39	1/156	3/158	4/314
45	2/156	3/158	5/314
51	1/156	4/158	5/314
52	22/156	22/158	44/314
53	4/156	7/158	11/314
56	2/156	4/158	6/314
58	47/156	49/158	96/314
66	4/156	2/158	6/314
68	4/156	4/158	8/314
Multiple infection excluding 16 or 18	31/156	18/158	49/314

LEEP, loop electrosurgical excision procedure; HR, high risk; HPV, human papillomavirus; HDC, HPV DNA chip test.

and 446 patients (60.5%) aged 36–45 years. High-grade CIN was confirmed by histology; 125 patients had CIN2, and 612 patients had CIN3. A total of 737 patients were enrolled with a median follow-up time of 3.5 years. Of the 737 patients, 36 (4.9%) developed recurrence during the follow-up period; histology revealed 6 cases of CIN2 (24.3%) and 30 cases of CIN3 (75.7%). The mean lag time between LEEP and the diagnosis of recurrent disease was 14.8 months (range, 6–48 months). Of the 737 patients included in the analysis, 360 patients received quadrivalent HPV vaccination and 377 patients did not receive quadrivalent HPV vaccination after LEEP for CIN2–3.

Baseline demographic characteristics were very much similar between patients in the vaccination and non-vaccination groups with respect to age, cytologic abnormalities, CIN grade, cone margin involvement, and positive endocervical cytology at the time of LEEP. Baseline HPV positivity to HPV 16 or 18 was not significantly different in the group of patients with vaccination and non-vaccination ($P = 0.790$) (Table 1).

The concordance and discordant results between the 2 HPV tests are summarized in Table 2. The overall agreement between the 2 tests was 98.5%, with a kappa value of 0.659. Of 719 HR-HPV-positive specimens by the HC2, the HDC was positive in 715 specimens (99.4%). Among 18 patients who were negative by the HC2, the HDC was negative in 11 patients (61.1%). When the HDC was compared to the HC2, discordant results were observed among 11 (1.5%) patients, comprising 4 HC2-positive/HDC-negative and 7 HC2-negative/HDC-positive results. Genotyping of 7 HC2-negative/HDC-positive cases revealed 3 HPV-53, 2 HPV-66, 1 HPV-16, and 1 HPV-18.

Table 2
The level of concordance between HR-HPV tests.

	No. of specimens (%) with HDC[a]		Total no. of specimens (%)
	Negative	Positive	
HC2[a]			
Negative	11	7	18 (2.4)
Positive	4	715	719 (97.6)
Total	15 (2.0)	722 (98.0)	

HR, high risk; HPV, human papillomavirus; HC2, hybrid capture II test; HDC, HPV DNA chip test.
[a] Absolute agreement = 98.5%, kappa = 0.659 ($P < 0.001$). Agreement between tests was assessed by Cohen's kappa statistic. *P* value was calculated using McNemar's test.

The distribution of prevalent HR-HPV genotypes by the HDC at the time of LEEP is presented in Table 3. HPV DNA was found in 98.0% (722/737) of the total cases. 55.4% (408/737) of patients with CIN2–3 related to HPV types 16 or 18 and 42.6% (314/737) were infected with other HR-HPV types at baseline by HDC. Additionally, most patients positive to vaccine-type HPV DNA were positive to only one HR-HPV type (22 were infected with exactly two vaccine types).

Analysis of populations to determine the impact of quadrivalent HPV vaccine after LEEP for CIN2–3 is shown in Fig. 1. Of 360 patients in the vaccination group, 9 (2.5%) developed recurrence during the follow-up period. Five (4 HPV-16 and 1 HPV-18) of 197 patients (2.5%) were related to HPV 16 or 18, but 4 of 163 patients (2.5%) who were unrelated to HPV 16 or 18 developed recurrence. Four of 5 patients related to vaccine HPV types had cone margin involvement, and 2 of 4 patients unrelated to vaccine HPV types had cone margin involvement. Of 377 patients in the non-vaccination group, 27 (7.2%) developed recurrence during the follow-up period. Eighteen (13 HPV-16 and 5 HPV-18) of 211 patients (8.5%) were related to HPV 16 or 18, but 9 of 166 patients (5.4%) who were unrelated to HPV 16 or 18 developed recurrence. Thirteen of 18 patients related to vaccine HPV types had cone margin involvement, and 2 of 9 patients unrelated to vaccine HPV types had cone margin involvement. With regard to patients related to vaccine HPV types, the non-vaccination group had a significantly higher recurrence rate than the vaccination group (8.5% and 2.5%, respectively; $P < 0.05$). Regarding patients unrelated to vaccine HPV types, the non-vaccination group had a slightly higher recurrence rate than the vaccination group, but recurrence rates were not significantly different between patients in the non-vaccination and vaccination groups (5.4% and 2.5%, respectively; $P = 0.257$). All 36 patients who developed recurrence tested positive for the same HPV genotype as the type before LEEP. None of the 22 patients who were positive to both HPV 16 and 18 developed recurrence.

Patients with recurrent disease did not differ from cured patients with respect to age, previous cytologic abnormalities, and CIN grade at the time of LEEP. Cone margin involvement, positive endocervical cytology, and non-recipients with quadrivalent HPV vaccine were associated with a significantly higher risk of recurrent disease ($P < 0.01$, Table 4).

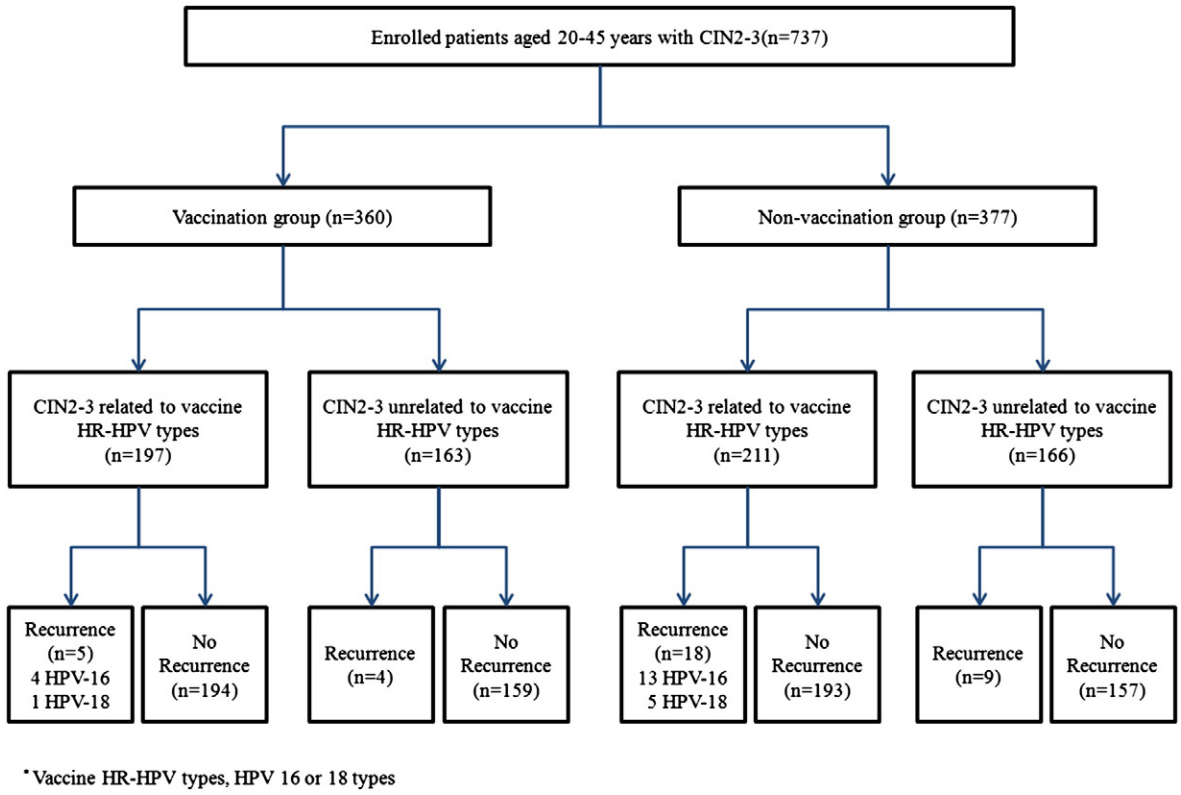

Fig. 1. Patient outcomes.

Based on the multivariate Cox regression model, the hazards ratio (HR) was adjusted for covariates. The risk of recurrence was higher for patients who did not receive the vaccine (HR = 2.840; 95% confidence interval [CI], 1.335–6.042; $P < 0.01$), with cone margin involvement (HR = 4.869; 95% CI, 2.365–10.221; $P < 0.01$), and positive endocervical cytology involvement (HR = 3.102; 95% CI, 1.363–7.062; $P = 0.01$), as shown in Table 5.

Discussion

A previous study has demonstrated that LEEP is currently one of the most common techniques for conization; it was described to effectively eradiate CIN2–3 [3,4]. Although the natural history of untreated HPV infection is well known, the progress of HPV infections after successful treatment is poorly understood. Several reports have suggested that successful conization also effectively eradicates HPV infections in most patients with CIN [13,14], and that the persistence of high-risk HPV infection at follow-up is a significant predictor of residual or recurrent CIN after conization. A study showed that HR-HPV infection clearance after conization with clear resection margins was 92.6–95.7% at the 6 month follow-up [15,16]. In our previous study, 37 of 672 patients (5.5%) developed residual/recurrent disease, and those who developed residual/recurrent disease tested positive for the same HR-HPV genotype before and after LEEP. The same HR-HPV genotype by the HDC during the follow-up period had a sensitivity and negative predictive value of 100% for detecting residual/recurrent disease [17].

In the present study, 9 of 360 patients (2.5%) in the vaccination group developed recurrent disease and 27 of 377 patients (7.2%) in the non-vaccination group developed recurrent disease. All 36 patients who developed recurrence tested positive for the same HR-HPV genotype by HDC.

The quadrivalent HPV vaccine is already proven for its benefits in women and girls aged 9–26 years [8,9]. In a recent study, the quadrivalent HPV vaccine was efficacious in women aged 24–45 years who were not infected with the relevant HPV types at enrolment [11]. The quadrivalent HPV vaccine is effective in women up to the age of 45, whereby prophylactic vaccine efficacy against diseases related to vaccine HPV types was 92.4% (49.6% to 99.8) [18]. Recently, Joura et al. [12] reported that the vaccine was associated with a significant reduction in the risk of any subsequent high grade disease of the cervix by 64.9% (20.1% to 86.3%). This study was an ad hoc analysis of FUTURE I and II that the trials were not designed or powered to evaluate the effect of vaccination after cervical surgery or diagnosis. Women with a prior history of HPV related disease were excluded from enrollment, so this study could not directly measure the vaccine's impact on women who had undergone treatment before vaccination, since all women in that study had been vaccinated before treatment.

No study has been done on the effect of prophylactic vaccination given after treatment of CIN2–3 in women who missed the opportunity of vaccination before developing the disease. Therefore, the aim of this study was to determine whether the vaccination with quadrivalent HPV vaccine among patients aged 20–45 years who had surgical treatment for CIN2–3 is effective in protecting the recurrence of the disease.

This study was able to demonstrate statistically significant efficacy against recurrent CIN2–3 in patients aged 20–45 years. Vaccination significantly reduced recurrent CIN2–3 after surgical treatment. In patients related to vaccine HPV types, the non-vaccination group had a significantly higher recurrence rate than the vaccination group (8.5% and 2.5%, respectively; $P < 0.05$). In patients unrelated to vaccine HPV types, the non-vaccination group had slightly higher recurrence rate than the vaccination group, but recurrence rates were not significantly different between patients in the non-vaccination and vaccination groups (5.4% and 2.5%, respectively; $P = 0.257$). Base on the multivariate Cox regression model, vaccination with the quadrivalent HPV vaccine among patients aged 20–45 years after LEEP for CIN2–3 was effective in preventing recurrent disease ($P < 0.01$), even after evaluation of other factors, including positive endocervical cytology and positive cone margin. The implications of these findings are relevant to the

Table 4
Patient characteristics according to recurrence.

	No recurrence N = 701	Recurrence N = 36	*P*
Age (years)			0.689
Mean ± SD	36.70 ± 5.79	36.29 ± 6.35	
Range			
Initial cytology	20–45	24–45	0.354
ASCUS	105	3	
LSIL	68	2	
HSIL	528	31	
CIN at LEEP			>0.99
CIN2	119	6	
CIN3	582	30	
Cone margin			<0.01
Negative	586	15	
Positive	115	21	
Endocervical cytology			<0.01
Negative	674	27	
Positive	27	9	
Vaccination			<0.01
Recipients	351	9	
Non-recipients	350	27	

SD, standard deviation; 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.

construction of accurate recommendations for CIN2–3 patients who underwent LEEP.

The HDC is a newly developed biotechnology which may be applied for the detection and typing of HPV. The accuracy of the HDC for the detection and typing of HPV in cervical lesions by comparing the results of HPV DNA sequencing with the same samples was 257 of 282 cases (91.1%) [19]. In the current study, the degree of concordance between the HDC and the HC2 was 98.5% (Cohen's kappa, 0.659 [substantial agreement]). In our previous study involving the concordance of both HPV tests in patients with CIN2–3, the overall agreement between the 2 tests was 97.3%, with a kappa value of 0.815 (near-perfect agreement) [17]. Of 652 HPV-positive specimens by the HC2, the HDC was positive in 644 specimens (98.8%). Among 20 specimens that were negative by the HC2, the HDC was negative in 10 specimens (50.0%). The HPV detection rate determined by the HDC was comparable to that determined by the HC2 in patients with invasive cervical cancer and its precursors.

The current study was the first study to examine vaccinated patients who underwent treatment for CIN2–3. Despite the limitations of analyzing the retrospective data, our results indicate that vaccination with the quadrivalent HPV vaccine among patients aged 20–45 years who had surgical treatment for CIN2–3 is effective in preventing recurrent CIN2–3. After treatment for CIN2–3, women are at increased risk for recurrent disease in the long run, and those with vaccination had a significantly reduced risk of recurrent CIN2–3. Our analysis also showed that vaccination after treatment of CIN2–3 significantly reduced the risk of recurrence in patients related to vaccine HPV types (types 16 and 18) and that the quadrivalent HPV vaccine is the prophylactic vaccine for recurrent CIN2–3.

A randomized, placebo-controlled, double-blind, and efficacy study is required to confirm that vaccination prevents recurrent disease in patients who underwent treatment for CIN2–3 and to define the appropriate time to start the first dose of quadrivalent HPV vaccination after LEEP for women who missed the opportunity of vaccination before developing the disease.

Role of the funding source

This study was supported by a grant from the Department of Obstetrics and Gynecology, Chonnam National University Medical School without influence regarding the design and data collection, analysis, and interpretation.

Table 5
Progression-free survival analysis by the Cox model.

	Hazards ratio (95% CI)	*P* value
Cone margin		
Positive versus negative	4.869 (2.365–10.221)	<0.01
Endocervical cytology		
Positive versus negative	3.102 (1.363–7.062)	<0.01
Vaccination		
Non-recipients versus recipients	2.840 (1.335–6.042)	<0.01

CI, confidence interval.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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