RESEARCH ARTICLE

Application of HPV DNA Testing in Follow-up after Loop Electrosurgical Excision Procedures in Northern Thailand

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Abstract

Background: HPV DNA testing has been recently introduced as an adjunct test to cytology in the follow-up of patients after treatment for cervical lesions using the loop electrosurgical excision procedure (LEEP). The aim of this study was to evaluate the role of HPV testing in the detection of persistent or recurrent disease after LEEP in patients with cervical epithelial lesions in northern Thailand. Materials and Methods: Patients who underwent LEEP as a treatment for histological low-grade (LSIL) or high-grade squamous intraepithelial lesion (HSIL) or worse at Chiang Mai University Hospital between June 2010 and May 2012 were included. Follow-ups were scheduled at 6-month intervals and continued for 2 years using co-testing (liquid-based cytology and Hybrid Capture 2 [HC2]) at 6 months and 24 months and liquid-based cytology alone at 12 and 18 months. Results: Of 98 patients included, the histological diagnoses for LEEP included LSIL in 16 patients, and HSIL or worse in 82 patients. The LEEP margin status was negative in 84 patients (85.7%). At follow-up, 10 patients (10.2%) had persistent/recurrent lesions; 4 among LSIL patients (25.0%) and 6 in the group with HSIL or worse (7.3%). Only 2 of 82 patients (2.4%) with HSIL or worse diagnoses had histological HSIL in the persistent/recurrent lesions. Using histologically confirmed LSIL as the threshold for the detection of persistent/recurrent disease, cytology had a higher sensitivity than HC2 (90.0% versus 70.0%). At the 6-month follow-up appointment, combined cytology and HC2 (co-testing) had a higher sensitivity in predicting persistent/recurrent disease (80.0%) compared with that of cytology alone (70.0%) and HC2 (50.0%). Conclusions: After LEEP with a negative surgical margin, the rate of persistent/recurrent lesions is low. The addition of HPV testing at the 6-month visit to the usual cytology schedule may be an effective approach in the follow-up after LEEP.

Keywords: Cervix - squamous intraepithelial lesion - LEEP - follow-up - HPV test - Thailand

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Introduction

Human papillomavirus (HPV) is a necessary cause of almost all cervical cancers and precancerous lesions. Cervical cancer screening using cytology and/or high-risk HPV DNA testing helps detect precancerous lesions or early cervical cancers that can be surgically treated (Siriaunkgul et al., 2014). Cervical conization with the loop electrosurgical excision procedure (LEEP) has an important role in both diagnosis and treatment of cervical epithelial lesions by the removal of them for a thorough pathologic examination (Wright et al., 2003; Sangkarat et al., 2014).

In patients who undergo conization as a conservative treatment for cervical epithelial lesions, a follow-up evaluation is important in order to identify the presence of persistent or recurrent disease. Cytology (±colposcopy) is the standard method used in the follow-up after conization (Wright et al., 2003). Recently, HPV testing has been introduced as an additional test in combination with cytology in the patients' follow-up, and many studies on this topic have been published (Nobbenhuis et al., 2001; Paraskevaidis et al., 2004). In previous studies, post-treatment HPV testing has been reported to have a high sensitivity and negative predictive value in the prediction of persistent/recurrent lesions, which suggests a possibility that HPV testing may help reduce the number of follow-up visits (Houfflin Debarge et al., 2003; Alonso et al., 2006; Kitchener et al., 2008; Jeong et al., 2009; Lubrano et al., 2012; Ryu et al., 2012). However, there is very limited information regarding the application of HPV testing in patient follow-up after LEEP in the populations of Southeast Asia or developing countries, where cervical cancer incidence is high (Nessa et al., 2014).

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The aim of this study is to evaluate the role of high-risk HPV DNA testing in the detection of persistent or recurrent disease after LEEP in patients with cervical epithelial lesions in northern Thailand.

Materials and Methods

The study was approved by Institutional Ethics Committee. Patients who underwent LEEP at Chiang Mai University Hospital between June 2010 and May 2012 were invited onto the study. Informed consent was obtained. In this institution, LEEP was carried out in all women with cytology results or cervical biopsy histology of high-grade cervical epithelial lesions. For women with minor cervical cytologic abnormalities, LEEP was performed if the initial work-up results suggested the presence of high-grade lesions. However, LEEP following colposcopy without intervening histological diagnosis may be performed in women with minor cytologic abnormalities, whose colposcopic findings were suggestive of high-grade disease. In most patients, endocervical curettage was also performed after LEEP. In patients with a positive surgical margin for high-grade epithelial lesions, a repeat LEEP (re-LEEP) was justified when feasible. The histological diagnosis in each patient was based on the most severe lesion detected in either the LEEP/re-LEEP specimen or the cervical biopsy prior to LEEP. In patients who underwent a re-LEEP, the margin status was determined in the re-LEEP specimens.

Inclusion criteria were patients with a histological diagnosis of squamous intraepithelial lesion (SIL), either low-grade (LSIL) or high-grade (HSIL), or microinvasive carcinoma who underwent LEEP conization as a treatment with the aim of achieving a complete removal of the epithelial lesions. Exclusion criteria were patients who had no histological confirmation of SIL in LEEP or prior cervical biopsy, patients who underwent LEEP as a diagnostic test prior to hysterectomy, or patients with incomplete follow-up and with no persistent/recurrent lesion detected.

Follow-ups were scheduled at 6-month intervals during the 2 years after LEEP (6, 12, 18, and 24 months), using co-testing (HPV test and liquid-based cytology) at 6 month and 24 months and cytology alone at 12 and 18 months. Patients who had negative test results in all follow-up visits during the 2-year period returned to the annual screening program. Patients who had cytological abnormalities or HPV positivity at 24 months received a colposcopic evaluation. During the colposcopy, a cervical biopsy was performed when any abnormality was detected. If the transformation zone was not visible or no abnormality was identified, endocervical sampling by brushing cytology or curettage was obtained. The presence of histologically confirmed epithelial lesions (HSIL or LSIL) within the first 6 months was considered as persistent disease and after 6 months as recurrent disease (Sangkarat et al., 2014).

In each follow-up visit, a cervical specimen was collected using a plastic spatula and a cytobrush. The cervical samples were transferred from the collecting devices into PreservCyt solution (Cytyc Corporation, Boxborough, MA, USA) for liquid-based cytology preparation (ThinPrep: Hologic, Marlborough, MA, USA) and for HPV testing (Hybrid Capture 2 [HC2]: Qiagen, Hilden, Germany). According to the manufacturer's information, HC2 is designed to detect 13 high-risk HPV genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68). Cervical cytology was reported according to the 2001 Bethesda System. Cytology was considered as abnormal when the result was at least atypical squamous cells of undetermined significance (ASC-US). A positive HC2 test is defined with a quantitative threshold from a relative light unit/positive control (RLU/PC) ratio ≥1.0.

The data including patient age, LEEP histological diagnosis, LEEP margin status, endocervical curettage results at LEEP, and the results of HC2 and cytology at follow-up were analyzed using STATA version 11 (StataCorp LP, College Station, TX, USA). Comparison of the difference between variables was evaluated using Fisher Exact test. A p value <0.05 was considered statistically significant. Accuracy values of the follow-up testing methods in the prediction of persistent/recurrent disease were calculated.

Results

There were 98 patients included in this study. A further 4 patients were excluded due to incomplete follow-up HPV testing and/or cytology at 24 months. The histological diagnoses included LSIL in 16 patients, HSIL in 80 patients, and microinvasive squamous cell carcinoma in 2 patients (stage IA1: extent of stromal invasion <1 mm and without lymphovascular space invasion). The mean patient age was 45.3 ± SD 9.2 years (45.1 years for LSIL and 45.4 years for HSIL or worse). Immediate re-LEEP was performed in 14 patients. The LEEP margin status was negative in 84 patients (85.7%). The remaining 14 patients (14.3%) had either positive (12 patients) or uncertain/non-evaluable margin status (2 patients).

At follow-up, 10 patients (10.2%) had persistent/recurrent lesions (1 persistent, 9 recurrent); 6 from 82 patients (7.3%) after the diagnosis of high-grade lesions (HSIL or worse) and 4 from 16 patients (25.0%) after a diagnosis of LSIL. The persistent/recurrent lesions in 6 patients with HSIL or worse diagnosis were histologically identified as HSIL in 2 patients and LSIL in 4 patients. All 4 patients with persistent/recurrent disease after the LSIL diagnosis had LSIL histology in the recurrent lesions. Three patients who had persistent /recurrent disease underwent a subsequent hysterectomy after 12 months of follow-up. The two patients with stage IA1 squamous cell carcinoma in LEEP had negative follow-up testing (HPV and cytology) in all visits, without any clinical evidence of recurrence.

The rate of persistent/recurrent lesions among the patients with LSIL diagnosis was higher than that of the patients with HSIL or worse, with a marginal significance (25.0% vs 7.3%, p=0.055). Thus, data of both groups were separately analyzed for the factors predicting persistent/recurrent disease; those with HSIL or worse diagnosis (Table 1) and those with LSIL diagnosis (Table 2).

Among patients with HSIL or worse diagnosis, age

≤35 years and positive or uncertain margin status were significantly associated with a higher risk for persistent/recurrent disease (p=0.038 and p=0.029, respectively). At follow-up, positive HC2 (p=0.038), positive cytology (p=0.001), and positive combined test results (p<0.001) were significantly predictive of persistent/recurrent disease. At 6 months after LEEP, cytology and co-testing were also significantly predictive of persistent/recurrent disease (p<0.001, each), but HC2 alone was not (p=0.080). In the case of positive surgical margin status, the risk of persistent/recurrent disease was not significantly different between the margin types (endocervical versus ectocervical). Among the patients with LSIL diagnosis, HC2 positivity was the only variable that showed a significant association with persistent/recurrent disease (p=0.019).

The rate of positive HC2 was higher among the patients with a positive or uncertain LEEP margin status (5 of 14 or 35.7%) than those with a negative margin (14 of 84 or 16.7%), but the difference was not significant (p=0.138). One of 2 patients with persistent/recurrent HSIL had the cytology result of HSIL with a negative HC2 test (RLU/PC ratio of 0.84) at the 6-month follow-up appointment. Of 68 patients who were HC2-negative at 6 months and had negative cytology results within 24 months, only one patient (1.5%) had recurrent LSIL and this patient was HC2- positive at 24 months. Three patients who were HC2-positive but cytology-negative at 24 months had negative colposcopic findings and biopsy.

The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of HC2 and cytology in the prediction of persistent/recurrent lesions

Table 1. Comparison of Variables for Persistent/recurrent Disease in 82 Patients with a Diagnosis of HSIL or Worse

Variable	Total No. (%)	No. of patients with persistent/recurrent disease	No. of patients with persistent/recurrent high-grade lesions
Age			
≤35 years	12 (14.6)	3 (25.0%)*	0 (0%)
>35 years	70 (85.4)	3 (4.3%)	2 (2.9%)
Margin status			
Positive or uncertain	11 (13.4)	3 (27.3%)*	0 (0%)
Negative	71 (86.6)	3 (4.2%)	2 (2.8%)
Endocervical curettage			
Positive	4 (4.9)	1 (25.0%)	0 (0%)
Negative	72 (87.8)	4 (5.6%)	1 (1.4%)
Not available	6 (7.3)	1 (16.7%)	1 (16.7%)
HC2 at 6 months			
Positive	7 (8.5)	2 (28.6%)	1 (14.3%)
Negative	75 (91.5)	4 (5.3%)	1 (1.3%)
Cytology at 6 months			
Positive	7 (8.5)	4 (57.1%)***	2 (28.6%)
Negative	75 (91.5)	2 (2.7%)	0 (0%)
Combined HC2 and cytology at 6 months			
Positive	16 (19.5)	5 (31.3%)***	2 (12.5%)
Negative	66 (80.5)	1 (1.5%)	0 (%)
Any positive HC2			
Yes	12 (14.6)	3 (25.0%)*	1 (8.3%)
No	70 (85.4)	3 (4.3%)	1 (4.3%)
Any positive cytology			
Yes	17 (20.7)	5 (31.3%)**	2 (11.8%)
No	65 (79.3)	1 (1.5%)	0 (0%)
Combined HC2 and cytology			
Positive (any test)	23 (28.0)	6 (26.1%)***	2 (8.7%)
Negative	59 (72.0)	0 (0%)	0 (0%)
Total	82 (100)	6 (7.3%)	2 (2.4%)

*p<0.05; **p<0.005; ***p<0.001

Table 2. Comparison of Variables for Persistent/recurrent Disease in 16 Patients with a Diagnosis of LSIL

Variable	Total No. (%)	No. of patients with persistent/recurrent disease
Age		
≤35 years	1 (6.3)	1 (100%)
>35 years	15 (93.7)	3 (20.0%)
Margin status		
Positive or uncertain	3 (18.8)	2 (66.7%)
Negative	13 (81.2)	2 (15.4%)
Endocervical curettage		
Positive	0 (0)	0 (0%)
Negative	16 (100)	4 (25.0%)
HC2 at 6 months		
Positive	6 (37.5)	3 (50.0%)
Negative	10 (62.5)	1 (10.0%)
Cytology at 6 months		
Positive	5 (31.3)	3 (60.0%)
Negative	11 (68.7)	1 (9.1%)
Combined HC2 and cytology at 6 months		
Positive	7 (43.8)	3 (42.9%)
Negative	9 (56.2)	1 (11.1%)
Any positive HC2		
Yes	7 (43.8)	4 (42.9%)*
No	9 (56.2)	0 (0%)
Any positive cytology		
Yes	9 (56.2)	4 (44.4%)
No	7 (43.8)	0 (0%)
Combined HC2 and cytology		
Positive (any test)	9 (56.2)	4 (44.4%)
Negative	7 (43.8)	0 (0%)
Total	16 (100)	4 (25.0%)

*p<0.05

Table 3. Performance of the Tests Used in the Detection of Persistent/Recurrent Lesions at LSIL Threshold

	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
All patients (n=98)				
HC2 at 6 months	50	90.9	38.5	94.1
Cytology at 6 months	70	94.3	58.3	96.5
Co-test at 6 months	80	83	34.8	97.3
HC2 at 6 and 24 months	70	86.4	36.8	96.2
Cytology follow-ups	90	80.7	34.6	98.6
Combined tests	100	75	31.3	100
Patients with HSIL or worse (n=82)				
HC2 at 6 months	33.3	93.4	28.6	94.7
Cytology at 6 months	66.7	96.1	57.1	97.3
Co-test at 6 months	83.3	85.5	31.3	98.5
HC2 at 6 and 24 months	50	88.2	25	95.7
Cytology follow-ups	83.3	84.2	29.4	98.5
Combined tests	100	77.6	26.1	100

(using the threshold of LSIL) among all patients and among 82 patients with HSIL or worse diagnosis are presented in Table 3. Cytology had a higher sensitivity than HC2. At the 6-month follow-up appointments, co-testing had a higher sensitivity and NPV in predicting persistent/recurrent disease compared with that of cytology or HC2 alone; however, the specificity and PPV of co-testing were lower. Among the patients with HSIL or worse diagnosis, the performance of co-testing at the 6-month follow-up was comparable to that of the follow-up cytology until 24 months.

Among 16 patients with LSIL diagnosis, the sensitivity and NPV of HPV testing and cytology in predicting persistent/recurrent disease was high (100%), although HPV testing tended to have a higher specificity (75.0% vs 58.3%) and PPV (57.1% vs 44.4%). However, when compared to cytology alone, the combined results of HPV testing and cytology did not improve the detection of persistent/recurrent disease.

Discussion

Persistence or recurrence of SIL after LEEP conization was reported in 5-18% of patients (Houfflin Debarge et al., 2003; Jeong et al., 2009; Lubrano et al., 2012; Ryu et al., 2012). Several variables have been proposed to be predictive of persistent/recurrent disease including age, pre-treatment cytology, histological diagnosis in LEEP, surgical margin status, viral load in HPV testing (pre- or post-treatment), and pre-treatment HPV genotypes (Song et al., 2006; Jeong et al., 2009; Nam et al., 2009; Ryu et al., 2012). In many studies, the surgical margin status of LEEP has been reported to be one of the most important predictors of persistent/recurrent disease (Houfflin Debarge et al., 2003; Ryu et al., 2012). The difference in rate of positive LEEP margins (11-46%) (Houfflin Debarge et al., 2003) may partly contribute to the variation of recurrence rate after LEEP in different studies.

In patients treated for histological HSIL, the recurrence rate was reported to be higher in cases with a positive margin compared to those with a negative margin (27-36% versus 5-12%) (Houfflin Debarge et al., 2003; Alonso et al., 2006). The finding in our study (27.3% versus 4.2%) is in keeping with such reported rates. The rate of persistent/recurrent lesions as HSIL was only 2.4% in this study which was lower than in an earlier study (6.6%) (Ryu et al., 2012). The very low number of patients with HSIL who had persistent/recurrent disease in this study limits the statistical analysis for the predictive factors for persistence/recurrence.

The positive rate of HPV testing using HC2 after LEEP has been reported to be approximately 25% (Lubrano et al., 2012; Ryu et al., 2012). This rate tended to be higher in the cases with positive margin status than those with negative margin status (36-41% versus 17-31%), but the difference was not significant in either the current or previous studies (Houfflin Debarge et al., 2003; Song et al., 2006). The post-treatment HPV-positive rate may also be variable with different HPV detection methods (Lubrano et al., 2012).

In previous studies, HPV testing has been reported to be more sensitive than cytology in the detection of persistent/recurrent lesions after LEEP with a diagnosis of HSIL (Alonso et al., 2006; Ryu et al., 2012). HPV testing at 6-12 months has been found to have a very high sensitivity and NPV (both approaching 100%) in predicting persistent/recurrent disease (Alonso et al., 2006; Jeong et al., 2009; Lubrano et al., 2012). In the present study, the performance of co-testing at 6-months in the patients with HSIL or worse diagnoses was comparable to that of follow-up cytology until 24 months. However, the sensitivity of HPV testing in this study was lower than that previously reported. The low number of persistent/recurrent cases in this study limited the assessment of performance difference between different testing methods.

Follow-up after LEEP in patients with a diagnosis of LSIL is important as approximately 12% of these patients may have a progression of the disease to HSIL (Alonso et al., 2007). In the present and previous studies, persistence/recurrence rate after LEEP tended to be higher in the patients with a diagnosis of LSIL than those patients with HSIL diagnosis (25-33% versus 7-24%) (Alonso et al., 2007). This may be partly explained by the nature of SIL as HSIL is a neoplastic lesion of monoclonal origin whereas LSIL is more likely to represent a productive HPV infection (Ueda et al., 2003). Thus, a negative LEEP margin in the excision of HSIL may suggest a high possibility for a complete removal of the lesion. In patients with a diagnosis of LSIL, the sensitivity of HPV testing and cytology in predicting persistence/recurrence was similarly high. However, combining the result of HPV testing with that of the cytology did not improve the probablitiy in predicting persistent/recurrent disease in the patients with LSIL diagnosis, when compared with cytology alone.

Low compliance to follow-up is an important issue in the consideration of management of the patients. A previous study has noted this problem in patients in Thailand (Rattanalappaiboon et al., 2014). This factor supports the treatment policy to remove the entire lesion by achieving a negative conization margin to reduce the risk of persistent/recurrent disease, although the complication rates may be increased with extensive conization (Sangkarat et al., 2014). In the setting of this study where this policy for LEEP is applied, co-testing at 6 months after LEEP and cytology in the other visits may be an effective approach in the follow-up of patients after LEEP.

One of the limitations in this study is the absence of pre-treatment HPV testing for a comparison with post-treatment HPV results. Previous studies have shown a very high rate of positive pre-treatment HPV testing in patients with a diagnosis of HSIL (95-97%) and also that a high pre-treatment viral load is a risk factor for persistent/recurrent disease (Song et al., 2006; Nam et al., 2009). In the present study, one of 2 patients with persistent/recurrent HSIL was HPV-negative. In a previous study, 3 of 20 (15%) patients who had persistent/recurrent HSIL had a negative HC2 test (Lubrano et al., 2012). The finding suggests that HPV testing alone may not be an effective follow-up test and co-testing should be preferred. Another limitation in the present study is the

use of liquid-based cytology in follow-up, and the results may not be directly compared with the practice using conventional Pap cytology. In a recent large scale study, liquid-based cytology was found to increase the detection rate of ASC-US and LSIL in women younger than 40 years compared to conventional cytology; however, there is no significant difference in the detection of HSIL or worse (Sigurdsson, 2013).

In conclusion, the rate of persistent/recurrent disease after LEEP with a negative surgical margin is low, and the rate of persistent/recurrent high-grade lesions is very low. Combining HPV testing with cytology may increase the sensitivity in the detection of persistent/recurrent disease after LEEP conization, compared to cytology alone. The addition of HPV testing at the 6-month visit to the usual cytology schedule may be an effective approach in the follow-up after LEEP.

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