



Risk factors of persistent HPV infection after treatment for high-grade squamous intraepithelial lesion

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Abstract

Purpose To evaluate the risk factors associated with persistent high-risk human papillomavirus (HR HPV) infections in patients undergoing cervical excision for treatment of high-grade squamous intraepithelial lesion (HSIL).

Methods A retrospective cohort study included 160 patients who underwent cervical excision for treatment of HSIL between January 2014 and December 2014. The clinical characteristics, cervical cytology, and HPV test results were reviewed. Persistent HR HPV infections were identified within 6 months after treatment. The effects of various factors such as patient age, menopausal status, parity, HPV type, and histopathological results on persistent HR HPV infections were assessed using univariate and multivariate analyses.

Results The mean age of patients was 38.1 ± 11.5 years (range 18–86 years). Among them 148 (92.5%) had HR HPV infections, and persistent infections after surgical treatment were detected in 48 (32.4%) patients. Univariate logistic regression analysis showed that older age (> 50 years), short follow-up duration (< 3 months), and menopause were associated with persistent HR HPV infections. Multivariate analysis showed that menopausal status was the only significant independent predictor for HR HPV persistence after treatment (odds ratio, 5.08; 95% confidence interval, 1.93–13.36; $P = 0.001$).

Conclusions Persistent HR HPV infections were detected in approximately 30% of patients within 6 months after cervical excision for HSIL. Elderly patients with menopause are at increased risk of HR HPV persistence after treatment for HSIL.

Keywords Cervical intraepithelial neoplasia · Conization · HPV · Menopause

Introduction

The lifetime prevalence of human papillomavirus (HPV) infection is greater than 70% for sexually active women [1]. Approximately 90% of all infections are spontaneously cleared by the immune system within 2 years; however, 10–20% of infections persists and cause carcinogenesis [2]. Persistent infections with high-risk HPV (HR HPV) play a central etiological role in the development of cervical intraepithelial neoplasia (CIN) and invasive cancer [3–5]. Among the 20 types of HR HPV, HPV 16 and 18 are the most common types worldwide and account for approximately 70% of cervical cancers [6]. A recent study show

that HPV testing represents a more sensitive tool for primary cervical cancer screening compared to cytology [7].

High-grade squamous intraepithelial lesion (HSIL, CIN2/3) can progress to cervical cancer without proper treatment [8]. Cervical excisions such as cold knife conization and loop electrosurgical excision procedure (LEEP) are not only diagnostic procedures, but also appropriate treatments for HSIL [9, 10]. However, the disease can occasionally recur in patients even when the cervical lesion is successfully removed. Women with persistent HR HPV infections have an increased risk of disease recurrence, which can progress to cervical cancer [9]. Thus, determining risks of persistent HR HPV infection after cervical excision are useful for identifying women at a higher risk of disease recurrence. In the present study, the risk factors potentially associated with HR HPV persistence in women undergoing cervical excision for the treatment of HSIL were evaluated.

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Methods

In this retrospective cohort study, we investigated 160 patients who underwent cervical excision for treatment of HSIL from January 2014 to December 2014 at Cheil General Hospital & Women's Healthcare Center. Following approval by our institutional review board, the clinical characteristics, cervical cytology, and HPV test results were reviewed. Cases involving persistent HR HPV infection within 6 months of follow-up were identified. The grade of cervical cytology was determined based on the 2001 Bethesda system [11]. HPV detection was performed using the Anyplex II HPV 28 detection kit (Seegene Inc. Seoul, Korea). High-risk types are 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 69, 73, and 82, and low-risk types are 6, 11, 40, 42, 43, 44, 54, 61, and 70. The pathological diagnosis was based on colposcopy-directed biopsy.

Statistical analysis

Categorical variables were indicated as number and percentage and continuous variables as mean with standard deviation (SD). We used *t* test for continuous variables and Chi-square test for categorical variables. A logistic regression model was used to estimate odds ratio (OR) and 95% confidence interval (CI) to characterize the associations between HPV infection and clinical characteristics. A *P* value < 0.05 in the two-sided test was considered statistically significant. Statistical analyses were performed using IBM SPSS version 21.0 (SPSS Inc., Chicago, IL, USA).

Results

Patient clinical characteristics are presented in Table 1. The mean patient age was 38.1 ± 11.5 years. Most patients had abnormal cervical cytology results, and 72 (45.0%) patients had HSIL based on cytology. HR HPV infections were detected in 148 (92.5%) patients; 43 (29.1%) had HPV 16 or 18. The colposcopic biopsy results showed 64 (40.0%) patients had CIN2, and 86 (60.0%) CIN3. More patients received LEEP (61.9%) than cold knife conization (38.1%).

The HPV status was analyzed within 6 months after cervical excision. The mean follow-up duration of the HPV test was 83.8 ± 45.7 days (33–174 days). Forty-eight patients (30%) presented with persistent HR HPV infections. A large number of patients (112, 70%) showed HPV clearance after treatment. Analysis results of associated factors for HR HPV persistence after cervical excision are shown in Table 2. Mean age in the HPV persistence group was greater

Table 1 Clinical characteristics (*n* = 160)

Age, mean \pm SD (years, range)	38.1 \pm 11.5 (18–86)
Parity, mean \pm SD (range)	1.0 \pm 1.1 (0–5)
Menopause	
Yes	24 (15.0%)
No	136 (85.0%)
Cervical cytology	
Negative	2 (1.3%)
ASC-US	23 (14.4%)
LSIL	17 (10.6%)
ASC-H	46 (28.7%)
HSIL	72 (45.0%)
HPV status	
High-risk HPV	148 (92.5%)
HPV 16/18	43 (29.1%)
Other high-risk	105 (70.9%)
Negative	12 (7.5%)
Colposcopic biopsy	
CIN2	64 (40.0%)
CIN3	86 (60.0%)
Type of excision	
LEEP	99 (61.9%)
Cold knife conization	61 (38.1%)

SD standard deviation, ASC-US atypical squamous cells of undetermined significance, LSIL low-grade squamous intraepithelial lesion, ASC-H atypical squamous cells cannot exclude high-grade lesion, HSIL high-grade squamous intraepithelial lesion, HPV human papilloma virus, CIN cervical intraepithelial neoplasia, LEEP loop electro-surgical excision procedure

than that of the HPV clearance group (41.5 ± 14.8 years vs. 36.6 ± 9.5 years, *P* = 0.036). In addition, the number of menopausal women in the HPV persistence group was significantly greater than in the HPV clearance group (*P* = 0.001). We also evaluated the HPV persistence based on pre-excisional HPV type 16 or 18 infection. Initial HPV 16 or 18 infection was found in 43 patients. Of those, 15 (34.9%) patients had persistent infection after treatment. HPV 16 and HPV 18 were 11 and 3 patients, respectively. The one patient was HPV 16/18 coinfection. The analysis of surgical outcomes showed that the rate of CIN3 was lower in the HPV persistence group than the HPV clearance group, although without statistical significance (*P* = 0.093). Positive surgical margin was significantly lower in the HPV persistence group than the HPV clearance group (*P* = 0.014).

Univariate logistic regression was used to assess odds ratios (ORs) of individual risk factors. The analysis results are summarized in Table 3. Patients over 50 years of age had a significantly high risk of HPV persistence (OR 3.4; 95% CI 1.18–10.04; *P* = 0.024). In addition, menopausal status was significantly associated with HPV persistence (OR 4.7; 95% CI 1.87–11.86; *P* = 0.001).

Table 2 Analysis results for associated factors of HPV persistence after cervical excision

	HPV-positive, <i>n</i> = 48, (%)	HPV-negative <i>n</i> = 112, (%)	<i>P</i> value
Age, mean ± SD (years)	41.5 ± 14.8	36.6 ± 9.5	0.036
Parity, mean ± SD	1.2 ± 1.3	1.0 ± 1.0	0.381
Follow-up duration, mean ± SD	69.7 ± 43.7	89.9 ± 45.3	0.010
Menopause			
Yes	15 (31.3)	9 (8.0)	0.001
No	33 (68.8)	103 (92.0)	
Marriage			
Yes	32 (66.7)	80 (71.4)	0.575
No	16 (33.3)	32 (28.6)	
Type of excision			
LEEP	28 (58.3)	71 (63.4)	0.596
Cold knife conization	20 (41.7)	41 (36.6)	
Pre-excisional HPV type			
HPV 16 or 18	15 (31.3)	28 (25)	0.702
HPV 16	11	20	
HPV 18	3	7	
HPV 16/18 coinfection	1	1	
Other HR HPV's	33 (68.7)	72 (75)	
Post-excisional HPV 16/18			
HPV 16	11	–	
HPV 18	3	–	
HPV 16/18 coinfection	1	–	
Final pathology			
CIN2	20 (41.7)	30 (26.8)	0.093
CIN3	28 (58.3)	82 (73.2)	
Surgical margin			
Positive	5 (10.4)	32 (28.6)	0.014
Negative	43 (89.6)	80 (71.4)	

HPV human papilloma virus, SD standard deviation, LEEP loop electrosurgical excision procedure, CIN cervical intraepithelial neoplasia

Short-term follow-up within 3 months after treatment showed high risk of HPV persistence (OR 2.54; 95% CI 1.24–5.17; *P* = 0.011), and positive surgical margin showed low risk of HPV persistence (OR 0.3; 95% CI 0.11–0.80; *P* = 0.017).

To assess the compound effects of variables on the risk of HPV persistence, multivariate logistic regression analysis was performed (Table 4). Menopausal status was the only significant independent predictor of HPV persistence (OR 5.1; 95% CI 1.93–13.36; *P* = 0.001). No significant association between follow-up duration or pathological diagnosis and HPV persistence was observed.

Table 3 Results of univariate logistic regression analysis

Category	Odds ratio (95% CI)	<i>P</i> value
Age (years)		
≥ 51	3.4 (1.18–10.04)	0.024
41–50	0.7 (0.18–1.76)	0.325
31–40	0.9 (0.35–2.08)	0.852
≤ 30	1	
Follow-up duration		
< 3 months	2.5 (1.24–5.17)	0.011
3–6 months	1	
Menopause		
Yes	4.7 (1.87–11.86)	0.001
No	1	
Final pathology		
CIN3	0.5 (0.25–1.04)	0.065
CIN2	1	
Surgical margin		
Positive	0.3 (0.11–0.80)	0.017
Negative	1	

CI confidence interval, CIN cervical intraepithelial neoplasia

Table 4 Results of multivariate logistic regression analysis

Category	Odds ratio (95% CI)	<i>P</i> value
Follow-up duration		
< 3 months	2.1 (0.99–4.50)	0.053
3–6 months	1	
Menopause		
Yes	5.1 (1.93–13.36)	0.001
No	1	
Final pathology		
CIN3	0.5 (0.23–1.07)	0.072
CIN2	1	

CI confidence interval, CIN cervical intraepithelial neoplasia

Discussion

Cervical excision is considered a procedure that can effectively eradicate not only CIN lesions, but also HPV infection. In this study, despite removal of the entire lesion using cervical excision with negative margins, 43 (26.9%) patients had persistent HPV infection. Several previous studies on HPV persistence after CIN treatment reported rates of HPV persistence at 6 months ranging from 14.3 to 33.0% [12–16]. A prospective study showed that HR HPV persistence after conization was an independent risk factor for HSIL recurrence [17]. The recurrence after treatment mostly occurred in patients with persistent HR HPV infections, regardless of the cervical cytology results [18, 19].

Therefore, evaluation of HR HPV infection on follow-up is essential since women treated for precancerous lesions are at risk of disease recurrence.

Factors associated with persistent HR HPV included age, lesion grade, resection margin status, HPV 16 infection, and viral load [12, 15–21]. However, in previous studies, the results differed for each risk factor. In the present study, univariate analysis revealed that age, menopause, and short-term follow-up duration (< 3 months) were significant predictors of HR HPV persistence after treatment (Table 3). Most HPV infections are cleared gradually after cervical excision. Persistent HPV infections were observed in 45.6%, 14.3%, 6.3%, 2.2%, 1.5%, and 1.1% of patients at 3, 6, 9, 12, 18, and 24 months, respectively, after LEEP [12]. Therefore, relatively high prevalence of HPV infection presents on the short-term follow-up test after treatment. Persistence of HPV infection after treatment was more common in older women, and advanced age was a significant risk factor for persistent infection in previous studies [18, 21]. In the present study, grade of lesion, HPV 16 or 18 infection, and type of excision were not associated with HPV persistence after treatment. The involved surgical margin was considered a risk factor for HPV persistence and disease recurrence [19]. We found minimal evidence to support this result in our study population and conversely observed that positive margin was a significant predictor of HPV clearance. Our results are similar to a previous cohort study by Costa et al. They analyzed the predicting factors associated with HPV clearance after CIN treatment in a cohort of 252 women, and showed the result that the involvement of endocervical margin was a significant independent predictor of HPV clearance ($P=0.001$) [18]. Further prospective studies with large scale would be needed to verify these results.

Based on the results from multivariate analysis, menopausal status is the most significant independent risk factor of persistent HPV infection. Although previous studies have reported that persistent HPV infection was associated with older age after treatment, an association with menopause was not observed. The relative prevalence of HPV is strongly associated with sexual activity after treatment, but this exposure is apparently rare, especially for elderly women. Nevertheless, high prevalence of HPV infection after treatment in menopausal women may occur due to reactivation or impaired clearance in older women following hormonal changes and immunological impairment. Persistent HPV infection in elderly women shows that cellular immune function may be strongly associated with the length of HPV persistence [22]. A previous study with 206 postmenopausal women after treatment of HSIL reported that the persistence rate of HR HPV infection was 20.4%, and the recurrence rate (12.6%) was higher than in younger women [23]. Therefore, menopausal women with persistent HR HPV infection after

treatment should be closely followed because they are at increased risk of disease recurrence.

This study had several limitations. First, the relatively small sample size and our incomplete understanding of other potential factors (active sexual life, viral load, smoking history) limit the interpretation of our data. Second, data on whether the initial infection or reinfection is associated with new sexual partners are lacking. Further prospective studies with long-term follow-up data are necessary to evaluate the clinical implications for these risk factors and disease recurrence. In conclusion, the results of this study confirmed the important predictive value of menopause based on univariate and multivariate analyses. Elderly patients with menopause are at increased risk of persistent HR HPV infection after treatment for HSIL and should receive intensive follow-up after treatment for early detection of recurrent disease.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval The Institutional Review Board approved the study. All procedures performed in this study were in accordance with the ethical standards of the institution and with the 1964 Helsinki Declaration and its later amendments. For this type of study, formal consent is not required.

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