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Original Article

Pathological changes of high-risk human papillomavirus-positive cervical lesions after surgery and analysis of risk factors for persistent positivity

Zhenli Tang, Yueqin Tao, Demei Xin, Jingjing Qin

Department of Obstetrics, Gynecology and Reproductive Medicine, Beijing Chuiyangliu Hospital, Beijing 100022, China

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Abstract: Objective: To investigate the postoperative pathological changes and risk factors for persistent positivity of high-risk human papillomavirus (HPV) in cervical lesions. Methods: A total of 186 female patients who underwent either outpatient and inpatient loop electrosurgical excision procedure (LEEP) or cold-knife conization for CIN II, CIN III of the cervix were included in this study. Postoperative pathological changes were analyzed, and high-risk HPV (HR-HPV) infection and persistent positivity were assessed at 3, 6, 12, and 24 months after surgery. Patient characteristics, including age, parity, endocervical glandular involvement, HPV subtypes, and type of conization, were recorded. Factors associated with persistent HR-HPV positivity after surgery were analyzed. Results: The concordance rate between colposcopically directed biopsy and postoperative cervical conization pathology was 77.9%, with a Kappa value of 0.537. During follow-up, persistent HPV infection was observed in 101, 83, 46, and 42 patients at postoperative 3, 6, 12, and 24 months, respectively. Logistic regression analysis identified CINIII pathology, viral load ≥1,000 copies/mL, endocervical glandular involvement, and LEEP as independent risk factors for persistent HR-HPV infection after surgery. Conclusions: Patients with higher pathological grade, elevated viral load, and endocervical glandular involvement are at increased risk of persistent HR-HPV infection after surgery for cervical lesions. Postoperative cervical cancer screening should remain a priority, as some patients remain positive for HR-HPV after LEEP.

Keywords: High-risk type, human papillomavirus-positive, cervical lesions, persistent positivity, risk factors

Introduction

Cervical cancer (CC) is the second most common cancer and the second leading cause of cancer-related death among women of childbearing age in the world [1]. The incidence of CC ranks second among fatal tumors in developing countries and tenth in developed countries [2]. Human papillomavirus (HPV) infection is a major etiological factor for cervical intraepithelial neoplasia (CIN) and CC. During active papillomavirus infection, infected basal cells replicate and expand locally [3]. Persistent HPV infection can progress to squamous intraepithelial lesions (SILs), which are classified into CIN I, CIN II, and CIN III according to the degree of epithelial involvement. High-risk HPV (HR-HPV) is implicated in 99% of CC cases [4], with over 200 HPV genotypes identified to date [5].

According to epidemiological studies, approximately 75% of the U.S. population aged 15-50 years will be infected with HPV in their lifetime, with 60% of infections being transient, 10% persistent, 4% leading to mild cytologic changes, and only 1% causing clinical cytological impairment [6]. Persistent infection with approximately 15 HR-HPV genotypes constitutes a major risk factor for CC, with HPV 16 and HPV 18 accounting for approximately 70% of the total cases [7]. Although HPV-16 and 18 are predominant worldwide, other HR-HPV genotypes (e.g., HPV-16 and HPV-33) also paly significant roles in CC development [8]. For instance, HPV-16 and HPV-33 have recently

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been reported to be the single most common HR-HPV genotypes in CIN II+ populations [9]; HPV-35 has reported to be one of the most predominant genotypes of CIN III in women in South Africa, second only to HPV-16 [10]; and the most common carcinogenic HPV subtypes in southwest China include HPV-16, 58, and 33 [11]. Therefore, apart from HPV-16 and 18, other oncogenic HR-HPV subtypes warrant attention, highlighting the importance of HPV screening for CC prevention and early detection.

Most HPV infections are transient and resolve spontaneously within a few years; however, 10-20% of HPV infections persist [12]. Persistent HR-HPV infection is strongly associated with CIN, which represents a prerequisite for the development of cervical precancerous lesions to CC [13]. Precancerous lesions include histological abnormalities, such as atypical squamous cells of undetermined significance (ASCUS) and low-grade SIL (LSIL/CIN I), which can progress to moderate dysplasia (CIN II) or severe dysplasia/carcinoma in situ (CIN III/CIS) [14]. The treatment of HPV-induced lesions depends on lesion severity. ASCUS and CIN I generally do not require immediate treatment due to high spontaneous regression rates; whereas high-grade lesions (HSIL/CIN II-III) often require conservative surgical procedures, including ablation or excision, to remove affected cervical tissue [15]. Cervical conization is a widely used approach for diagnosis and treatment of CIN. However, women with HSIL (CIN II/III) remain at increased risk of recurrence of CIN II or greater (CIN II+) after loop electrosurgical excision procedure (LEEP), particularly in HR-HPV-positive patients [16, 17]. Prospective studies have shown that HPV infections comprise both sporadic and persistent types, with persistence resulting from incomplete viral clearance over time [18].

Persistent HPV infection is a special concept. Most scholars define persistence as an infection that results in two consecutive positive HPV DNA tests with an uncertain time interval [19]. Others define persistence base on clearance time or as detection of the same virus strain in a patient for more than 9 months [13]. Despite advances in screening, HPV remains a global health burden due to the absence of treatments that can eradicate the virus or prevent its continued existence. Therefore, the prognosis of HSIL patients after conization remains a significant clinical concern, as persistent HPV infection increases the risk of postoperative recurrence, making such patients a high-risk population for CC.

This study retrospectively analyzed clinical data from patients with CIN II and CIN III who underwent conization, aiming to evaluate the postoperative HPV infection prognosis and identify factors associated with persistent infection. We anticipate that this study may provide new insight into the management of high-risk post-surgical patients and reduce the risk of postoperative HSIL recurrence.

Data and methods

Research subjects

This retrospective study included 186 female patients with CIN II or CIN III who attended Beijing Chuiyangliu Hospital between January 2020 to January 2024. All patients underwent either outpatient or inpatient LEEP or cold-knife conization (CKC). This study was approved by the Medical Ethics Committee of Beijing Chuiyangliu Hospital.

Inclusion criteria: (1) Patients who underwent preoperative liquid-based ThinPrep cytologic test (TCT) and quantitative HPV subtyping (fluorescence quantitative PCR), with electronic colposcopy and biopsy pathology results as the diagnostic basis; (2) Patients positive for HR-HPV before surgery; (3) Postoperative pathology confirmed CIN II-III with negative incisional margin tissues; (4) Complete clinical and follow-up data available.

Exclusion criteria: (1) HR-HPV negative; (2) Postoperative pathological evidence of invasive cervix, vaginal, or vulvar carcinoma; (3) History of major cervical diseases or uterus-related operations; (4) Other malignant tumors or severe systemic diseases involving major organs (e.g., heart, liver, and kidneys); (5) Cognitive or psychiatric disorders; (6) Incomplete clinical or follow-up data.

Research methods

(1) Cervical exfoliated cell examination: Cervical exfoliated cells were collected using con-

ventional methods for TCT and HPV testing. Patients were instructed to avoid vaginal cleaning, medication, or sexual activity within 72 hours before sampling. The sampling was avoided during menstruation. Collected cells were placed in ThinPrep solution, processed, and stained using Pap staining. Slides were examined under a microscope, and cells were classified according to cervical cytopathology criteria, including ACSUS, SILs [low/high-grade (LSIL/HSIL)], normal or inflammatory, and squamous cell carcinoma (SCC). HPV was detected using the Roche Cobas 4800 HPV DNA detection system. Fourteen carcinogenic HR-HPV genotypes recommended by WHO were detected using real-time PCR: HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68. Of them, HPV16 and 18 were analyzed individually, while the remaining 12 types were labeled as other HR-HPV subtypes. A positive in either HR-HPV or TCT was considered screening-positive for cervical cancer or precancerous lesions. All results were independently reviewed by two experienced laboratory physicians (5-10 years of work experience); discrepancies were resolved by a senior laboratory physician.

(2) Colposcopy and cervical biopsy: The vaginal wall and cervical epithelium were examined under a colposcope, Acetic acid test and iodine test were performed to identify lesions. Localization biopsy was performed according to the 2017 ASCCP colposcopy standard. Concurrent cervical canal curettage was performed for patients with type III transformation zone, high-grade cytological abnormalities, or HPV16/18 infection.

(3) Cervical conization: LEEP or CKC was performed. Non-menopausal patients underwent surgery 3-7 days after menstruation. The lesion area was identified using the iodine test. The complete transformation zone and part of the cervical canal above the squamocolumnar junction (SCJ) were removed. The resection length was determined based on the transformation zone type: 7-10 mm for type I, 10-15 mm for type II, and 15-25 mm for type III. Cervical transformation zones are classified anatomically according to the visibility of the SCJ under colposcopy. Type I: SCJ completely visible beyond the external OS of the cervix; Type II: SCJ partially extends into the cervical canal, but can be fully exposed with gentle manipulation; Type III: SCJ completely within the cervical canal, not visible under routine colposcopy.

Endpoints

(1) Data collection: Clinical data such as patient age, age at menarche, parity, history of gynecological diseases, delivery mode, family history of CC, lesion grade, viral load (VL), lesion involvement quadrant, and HPV genotype were collected. Immunohistochemical results of Ki67 and p16/INK4a were also obtained. HPV viral load reflects the quantity of viral genomes per unit sample, typically expressed as copies per milliliter (copies/mL). Cervical canal exfoliated cells were collected using a specialized cervical brush and quantified via real-time quantitative polymerase chain reaction (PCR).

(2) Follow-up: Follow-ups were carried out at 3, 6, 9, 12, and 24 months after the operation to assess HPV infection status.

Statistical processing of data

Based on existing clinical research data, the persistent HPV infection rate at 24 months post-surgery ranges from approximately 5% to 15%, with most cases clustering around 10%. Under the framework of a single-sample proportion hypothesis test, assuming an expected persistent infection rate of 20% and a baseline value of 10%, the minimum required sample size was calculated as 137 cases at a significance level of $\alpha=0.05$ (two-tailed) and a statistical power of $1-\beta=0.8$.

SPSS 25.0 was used for data processing. The Kappa test was used to assess the consistency between colposcopically directed biopsy results and pathological findings. Continuous data, expressed as mean ± standard deviation, were compared using t-test. Count data were expressed as percentages and analyzed using chi-square tests. Binary logistic regression was applied to identify factors associated with persistent HPV infection. A *P* value <0.05 was indicative of statistical significance.

Results

General information of 186 patients

A total of 186 CIN II/III patients were selected as the study subjects. The median age was 45 years (range: 26-65 years), median gravidity

Table 1. General information of study subjects

Clinical features	Number of cases (n=186)
Age (years)	
≤40	86 (46.2)
>40	100 (53.8)
Ethnicity	
Han	148 (79.6)
Ethnic minorities	38 (20.4)
Educational level	
Bachelor degree or above	95 (51.1)
Junior college/high school or below	91 (48.9)
Gravidity	
≥3	122 (65.6)
<3	64 (34.4)
Parity	
≥2	67 (36.0)
<2	119 (64.0)
Pathological grading	
CIN II	110 (59.1)
CIN III	76 (40.9)
Preoperative HR-HPV subtype	
HPV 16/18	122 (65.6)
Other 12 HPV subtypes	64 (34.4)
Types of preoperative HR-HPV infection	
Single infection	117 (62.9)
Mixed infection	69 (37.1)
Endocervical glandular involvement	
With	96 (51.6)
Without	90 (48.4)
Viral load (copies/mL)	
1-100	100 (53.8)
100-1,000	48 (25.8)
≥1,000	38 (20.4)
Operation mode	
LEEP	113 (60.8)
CKC	73 (39.2)

Notes: CIN, cervical intraepithelial neoplasia; HPV, human papillomavirus; LEEP, loop electrosurgical excision procedure; CKC, cold-knife conization.

was 3 (range: 2-4), and median parity was 1 (range: 1-2). Among the patients, 110 cases were CIN II and 76 cases were CIN III, with single subtype infection and mixed subtype infection accounting for 62.9% and 37.1%, respectively (**Table 1**). **Figure 1** illustrates the immunohistochemical features of a HSIL: The lesion is characterized by diffuse, disorganized epithelial arrangement along the basement membrane, a high mitotic index, and the presence of atypical mitoses near the surface (**Figure 1A**). Ki-67 immunostaining shows diffuse, full-thickness positivity (**Figure 1B**), with a positive rate of about 50%. p16-INK4A immunohistochemical staining exhibits a diffuse, full-thickness pattern in both nuclei and cytoplasm (**Figure 1C**).

Pathological analysis after colposcopically directed biopsy and cervical conization

Among the 186 patients who underwent conization, colposcopically directed biopsy diagnosed CIN II in 117 cases and CIN III in 69 cases, whereas postoperative pathology revealed CIN II in 110 patients and CIN III in 76 patients. A total of 145 cases showed complete concordance between biopsy and postoperative pathology, accounting for 77.9%. The Kappa value of the consistency test between colposcopically directed biopsy and conization pathology was 0.537 ($P<0.05$), indicating moderate consistency (**Table 2**).

Postoperative persistent HPV infection in patients with HSILs

During the follow-up of the 186 patients after conization, persistent HPV infection was observed in 101 (54.3%), 83 (44.6%), 46 (24.7%), and 42 (22.6%) patients at postoperative 3, 6, 12, and 24 months, respectively. Notably, the TCT results were normal in all patients throughout the 24 months follow-up period (**Table 3**).

Univariate analysis of patients with persistent HPV infection 24 months after surgery

Among the 42 patients with persistent HPV infection at postoperative 24 months, univariate analysis revealed no significant differences in HPV persistence between patients with different ages, nationalities, gravidity, or parity ($P>0.05$); however, significant differences were observed among patients in terms of educa-

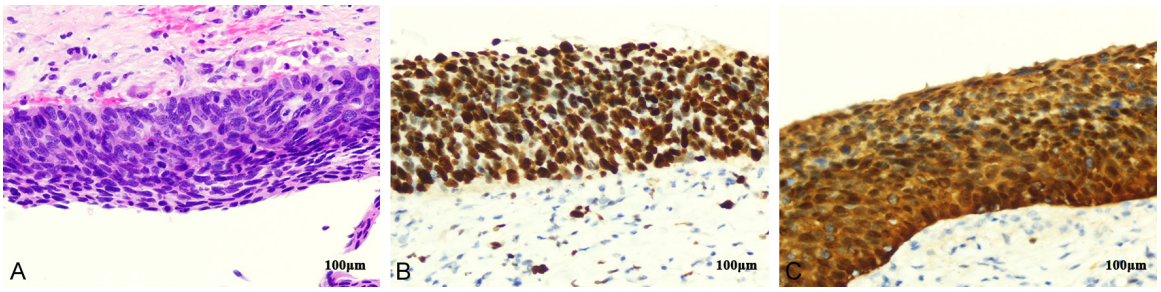

Figure 1. Histopathological and immunohistochemical staining of a HSIL. A: H&E staining; B: Ki67 expression; C: p16INK4a. Note: HSIL, high-grade squamous intraepithelial lesion.

Table 2. Concordance between colposcopically directed biopsy and pathological findings after conization

Colposcopically directed biopsy	Conization pathology		Total
	CIN II	CIN III	
CIN II	93 (50.0)	24 (12.9)	117 (62.9)
CIN III	17 (9.1)	52 (28.0)	69 (37.1)
Total	110 (59.1)	76 (40.9)	186 (100.0)

Notes: CIN, cervical intraepithelial neoplasia.

tional level, preoperative pathological grade, preoperative HR-HPV subtype, preoperative HR-HPV infection type, lesion involvement range, VL, and surgical mode (all $P<0.05$). Detailed results are shown in **Table 4**.

Logistic regression of patients with persistent HPV infection 24 months after surgery

Further multivariate logistic regression analysis was conducted on factors with statistical significance in the univariate analysis. The results demonstrated that CIN III pathology, endocervical glandular involvement (EGI), VL ≥1000 copies/mL, and LEEP were independent risk factors for postoperative persistent HPV infection at 24 months postoperatively (**Table 5**).

Discussion

HR-HPV infection is a major cause of cervical lesions. Most HPV infections are transient and resolve spontaneously, with only a small number of people progressing to CC. Low-risk HPV types generally induce benign lesions, such as verruca vulgaris and verruca plana, which are common in humans [20, 21]. Cervical precancerous lesions and cervical microinvasive carcinoma can be treated with conservative conization, minimizing both missed diagnoses of CC and overtreatment of precancerous lesions. However, the time to negative conversion of HR-HPV after conization in HSIL patients and the related factors affecting negative conversion are still inconclusive. Evidence suggests that HR-HPV can persist in the cervical epithelium through latent infection, resulting in persistent postoperative HPV infection, lesion recurrence, and involvement of adjacent vaginal tissue [22].

Immunohistochemical assessment of Ki67 and p16/INK4a has become an increasingly routine adjunctive tools in the diagnosis of CIN [23]. This combined testing has been widely incorporated into clinical practice, particularly when histological diagnosis is ambiguous or when distinguishing high-grade lesions is necessary. p16 is a tumor suppressor protein whose overexpression is typically triggered by inactivation of the Rb protein via HR-HPV (such as HPV16/18) E7 oncoproteins. In cervical tissue, diffuse strong p16 positivity (extending to the upper and middle epithelial layers) is a hallmark of HSIL/CIN II+ [24]. Ki67 is a nuclear antigen expressed in all phases of the cell cycle except G0, reflecting the degree of active cell proliferation [25]. Nevertheless, histopathological examination remains the gold standard for diagnosis. Also, identification and management of factors contributing to persistent postoperative HPV infection in HSIL patients are critical to reduce recurrence and improve long-term outcomes.

In this study, cervical conization was performed in CIN II/III patients, with some cases experiencing pathological escalation after the procedure. Pathological escalation after CIN III

Table 3. Number of patients with persistent HPV infection after surgery

Clinical features	3 months (n=101)	6 months (n=83)	12 months (n=46)	24 months (n=42)	χ^2	P
Age (years)					1.208	0.751
≤40	47 (46.5)	33 (39.8)	18 (39.1)	17 (40.5)		
>40	54 (53.5)	50 (60.2)	28 (60.9)	25 (59.5)		
Ethnicity					1.344	0.719
Han	82 (81.2)	71 (85.5)	38 (82.6)	37 (88.1)		
Ethnic minorities	19 (18.8)	12 (14.5)	8 (17.4)	5 (11.9)		
Educational level					3.043	0.385
Bachelor's degree or above	52 (51.5)	40 (48.2)	21 (45.7)	15 (35.7)		
Junior college/senior high school or below	49 (48.5)	43 (51.8)	25 (54.3)	27 (64.3)		
Gravidity					1.419	0.701
≥3	70 (69.3)	56 (67.5)	30 (65.2)	32 (76.2)		
<3	31 (30.7)	27 (32.5)	16 (34.8)	10 (23.8)		
Parity						
≥2	61 (60.4)	34 (41.0)	16 (34.8)	19 (45.2)		
<2	40 (39.6)	49 (59.0)	30 (65.2)	23 (54.8)		
Pathological grading					11.213	0.011
CIN II	56 (55.4)	43 (51.8)	17 (37.0)	16 (38.1)		
CIN III	45 (44.6)	40 (48.2)	29 (63.0)	26 (61.9)		
Preoperative HR-HPV subtype					2.202	0.532
HPV 16/18	63 (62.4)	50 (60.2)	31 (67.4)	22 (52.4)		
Other 12 HPV subtypes	38 (37.6)	33 (39.8)	15 (32.6)	20 (47.6)		
Type of preoperative HR-HPV infection					2.064	0.559
Single infection	58 (57.4)	40 (48.2)	25 (54.3)	20 (47.6)		
Mixed infection	43 (42.6)	43 (51.8)	21 (45.7)	22 (52.4)		
Endocervical glandular involvement					4.928	0.177
With	55 (54.5)	47 (56.6)	26 (56.5)	31 (73.8)		
Without	46 (45.5)	36 (43.4)	20 (43.5)	11 (26.2)		
Viral load (copies/mL)					7.451	0.281
1-100	42 (41.6)	22 (26.5)	12 (26.1)	12 (28.6)		
100-1000	23 (22.8)	27 (32.5)	14 (30.4)	10 (23.8)		
≥1000	36 (35.6)	34 (41.0)	20 (43.5)	20 (47.6)		
Operation mode					4.193	0.241
LEEP	78 (77.2)	61 (73.5)	33 (71.7)	37 (88.1)		
CKC	23 (22.8)	22 (26.5)	13 (28.3)	5 (11.9)		

Notes: CIN, cervical intraepithelial neoplasia; HPV, human papillomavirus; LEEP, loop electrosurgical excision procedure; CKC, cold-knife conization.

conization is related to stromal micro-infiltration, and endocervical glandular involvement is a high-risk factor for pathological progression after CKC, highlighting the need for careful assessment during diagnosis and treatment of cervical lesions. Although colposcopically directed biopsy and pathological diagnosis after cervical conization were largely consistent, the overall concordance was moderate, with several cases demonstrating pathological escalation or degradation after conization. During follow-up, persistent HPV infection was observed in 101, 83, 46, and 42 cases at 3, 6, 12, and 24 months after the operation, respectively. Univariate analysis indicated that persistent HPV infection after cervical surgery was not correlated with age, ethnicity, gravidity, or parity, but was significantly related to educational level, preoperative pathological grade, preoperative HR-HPV subtype, preope-

Table 4. Univariate analysis of patients with persistent HPV infection at postoperative 24 months

Clinical features	HPV negative (n=144)	HPV positive (n=42)	χ^2	P
Age (years)			0.724	0.395
≤40	69 (47.9)	17 (40.5)		
>40	75 (52.1)	25 (59.5)		
Ethnicity			2.426	0.119
Han	111 (77.1)	37 (88.1)		
Ethnic minorities	33 (22.9)	5 (11.9)		
Educational level			5.123	0.023
Bachelor's degree or above	80 (55.6)	15 (35.7)		
Junior college/senior high school or below	64 (44.4)	27 (64.3)		
Gravidity			2.700	0.100
≥3	90 (62.5)	32 (76.2)		
<3	54 (37.5)	10 (23.8)		
Parity			1.762	0.188
≥2	49 (34.0)	19 (45.2)		
<2	95 (66.0)	23 (54.8)		
Pathological grading			9.943	0.002
CIN II	94 (65.3)	16 (38.1)		
CIN III	50 (34.7)	26 (61.9)		
Preoperative HR-HPV subtype			4.195	0.041
HPV 16/18	100 (69.4)	22 (52.4)		
Other 12 HPV subtypes	44 (30.6)	20 (47.6)		
Type of preoperative HR-HPV infection			5.431	0.019
Single infection	97 (67.4)	20 (47.6)		
Mixed infection	47 (32.6)	22 (52.4)		
Endocervical glandular involvement			10.701	0.001
With	65 (45.1)	31 (73.8)		
Without	79 (54.9)	11 (26.2)		
Viral load (copies/mL)			26.122	<0.0001
1-100	88 (61.1)	12 (28.6)		
100-1000	38 (26.4)	10 (23.8)		
≥1000	18 (12.5)	20 (47.6)		
Operation mode			18.211	<0.0001
LEEP	74 (51.4)	37 (88.1)		
CKC	70 (48.6)	5 (11.9)		

Notes: CIN, cervical intraepithelial neoplasia; HPV, human papillomavirus; LEEP, loop electrosurgical excision procedure; CKC, cold-knife conization.

rative HR-HPV infection type, lesion involvement range, HR-HPV VL, and surgical mode. Further multivariate logistic regression analysis identified CIN III pathology, VL ≥1,000 copies/mL, EGI, and LEEP were independent risk factors for persistent postoperative HPV infection. In univariate analysis, both preoperative HPV subtype and infection types were significantly associated with persistent infection, reflecting that women may acquire multiple HPV genotypes over their lifetime [26]. Reported prevalence of multiple HPV infections among HPV-positive women ranges from 18.5% to 46% [27, 28]. Fife et al. [29] reported that infection with multiple HR-HPV genotypes often increases the severity of cervical diseases, while other reports provide controversial conclusions. Herrero et al. [30] indicated that multiple infections may prolong HPV persistence and elevate the risk of cervical disease. However, some studies found no significant impact [31, 32]. In this study, multivariate logistic regres-

Table 5. Multivariate logistic regression analysis of persistent HPV infection at postoperative 24 months

Variable	β	S.E	Wald	P	OR	95% CI
Education level (0= junior college/senior high school or below, 1= bachelor's degree or above)	-0.845	0.461	3.353	0.067	0.430	0.174-1.061
Pathological grading (0= CIN II, 1= CIN III)	1.175	0.454	6.687	0.010	3.238	1.329-7.888
Preoperative HR-HPV infection subtype (0= others, 1= HPV 16/18)	-0.598	0.453	1.741	0.187	0.550	0.226-1.337
Type of preoperative HR-HPV infection (0= single, 1= mixed)	0.812	0.477	2.899	0.089	2.252	0.884-5.734
Endocervical glandular involvement (0= no, 1= yes)	1.421	0.491	8.393	0.004	4.142	1.584-10.835
Viral load (copies/mL, control: 0=<100)	-	-	12.738	0.002	-	-
Viral load (1=100-1000)	0.392	0.565	0.482	0.488	1.480	0.489-4.477
Viral load (0=≥1000)	1.916	0.552	12.041	0.001	6.794	2.302-20.053
Operation mode (0=CKC, 1=LEEP)	2.642	0.718	13.542	0.000	14.035	3.437-57.313
Constant	-4.950	0.978	25.603	0.000	0.007	-

Notes: CIN, cervical intraepithelial neoplasia; HPV, human papillomavirus; LEEP, loop electrosurgical excision procedure; CKC, cold-knife conization.

sion did not support mixed HPV infection as an independent risk factor for persistent HPV infection.

Viral load (VL) is another key factor under debate. Most evidence indicates that higher HPV VL is positively correlated with the severity of CC and the likelihood of integration into host cells, serving as a potential marker for disease progression [33-36]. However, some studies have reported no significant association between HR-HPV VL and lesion severity [9]. Zhao et al. [37] conducted a 15-year prospective cohort study in China and demonstrated that women with an increased VL (15.3%) had a 38-fold higher risk of developing CIN II+ compared to HPV-negative women (0.4%). Nonetheless, the predictive value of VL may be genotype-specific. For example, a French cohort study [38] found that only the VL of HPV16 predicted CIN II+, whereas this association was not found in HPV18 or other genotypes, a pattern also noted in Chinese women [9]. Therefore, HSIL patients with high preoperative HR-HPV VL are at an increased risk of persistent postoperative infection and should be closely monitored postoperatively to prevent disease recurrence.

Furthermore, EGI was identified as a risk factor for persistent postoperative HPV infection, consistent with the findings of Baser et al. [39]. Glandular involvement at the lesion site indicates deep lesion penetration, high-grade multifocal lesions, and strong proliferative activity, which collectively increase the likelihood of residual disease after surgery. Finally, LEEP was also identified as an independent risk factor for persistent HPV infection after surgery. As a standard procedure for the treatment of CIN, cervical conization has significant clinical application value. However, conventional CKC is associated with a higher risk of infection, intraoperative and postoperative hemorrhage, and cervical insufficiency [40], although it ensures adequate resection with clear margins [40]. The tungsten wire loop used in LEEP is heated to high temperature, enhancing its flexibility. In addition, the cross-sectional diameter of the loop is only 0.1-0.3 mm, which generates tissue impedance upon contact with the lesion. In addition, the adhesion of heat-damaged tissue to the loop gradually increases resistance during excision and may cause subtle loop deformation, leading to a reduced cone height as the sample is tapered, resulting in insufficient LEEP cutting depth and an increase in the HPV persistent infection rate after LEEP conization [41]. Reported recurrence rates after CKC range from 1.4% to 2.2% [42, 43], compared with 7% to 14% after LEEP [44, 45]. Therefore, both CKC and LEEP have distinct advantages and should be selected based on the individual condition.

This study does have certain limitations. First, the relatively small sample size may not adequately represent patients across different age groups, lesion grades, and surgical approaches, leading to insufficient statistical power. This

prevents the precise identification of niche factors associated with persistent HPV positivity, such as specific HPV subtype combinations or the impact of rare immunodeficiencies on viral clearance. Second, the relatively short follow-up period limits assessment of long-term outcomes for persistent HPV infection. The natural course of HPV infection, including viral latency and reactivation after surgery, may span several years. A shorter follow-up period cannot fully capture disease progression at 3, 5, or more years postoperatively. Furthermore, insufficient exploration of potential influencing factors represents another significant limitation. Therefore, future studies should conduct standardized, multicenter, large-sample, long-term follow-up research to more accurately elucidate the clinical characteristics and pathogenesis of persistent HPV infection, providing more robust evidence-based medical support for clinical diagnosis and treatment.

Conclusion

Cervical conization effectively removes cervical lesions, clarifies lesion severity and extent, and guides subsequent treatment. However, some patients remains positive for HR-HPV after cervical conization, posing a risk for cervical cancer, emphasizing the importance of postoperative CC screening. In clinical practice, patient management should consider multiple factors comprehensively rather than in isolation.

Disclosure of conflict of interest

None.

Address correspondence to: Jingjing Qin, Department of Obstetrics, Gynecology and Reproductive Medicine, No 2, Chuiyangliu South Street, Chaoyang District, Beijing 100022, China. Tel: +86-67744566; E-mail: qinjingjing26@163.com

References

[1] Singh D, Vignat J, Lorenzoni V, Eslahi M, Ginsburg O, Lauby-Secretan B, Arbyn M, Basu P, Bray F and Vaccarella S. Global estimates of incidence and mortality of cervical cancer in 2020: a baseline analysis of the WHO global cervical cancer elimination initiative. Lancet Glob Health 2023; 11: e197-e206.

[2] Viveros-Carreño D, Fernandes A and Pareja R. Updates on cervical cancer prevention. Int J Gynecol Cancer 2023; 33: 394-402.

[3] Gonçalves CA, Pereira-da-Silva G, Silveira RCCP, Mayer PCM, Zilly A and Lopes-Júnior LC. Safety, efficacy, and immunogenicity of therapeutic vaccines for patients with high-grade cervical intraepithelial neoplasia (CIN 2/3) associated with human papillomavirus: a systematic review. Cancers (Basel) 2024; 16: 672.

[4] Clifford GM, Smith JS, Plummer M, Muñoz N and Franceschi S. Human papillomavirus types in invasive cervical cancer worldwide: a meta-analysis. Br J Cancer 2003; 88: 63-73.

[5] Van Doorslaer K, Li Z, Xirasagar S, Maes P, Kaminsky D, Liou D, Sun Q, Kaur R, Huyen Y and McBride AA. The papillomavirus episteme: a major update to the papillomavirus sequence database. Nucleic Acids Res 2017; 45: D499-D506.

[6] Chesson HW, Dunne EF, Hariri S and Markowitz LE. The estimated lifetime probability of acquiring human papillomavirus in the United States. Sex Transm Dis 2014; 41: 660-664.

[7] Welby S, Rosillon D, Feng Y and Borys D. Progression from human papillomavirus (HPV) infection to cervical lesion or clearance in women (18-25 years): natural history study in the control arm subjects of AS04-HPV-16/18 vaccine efficacy study in China between 2008 and 2016. Expert Rev Vaccines 2022; 21: 407-413.

[8] Ma X and Yang M. The correlation between high-risk HPV infection and precancerous lesions and cervical cancer. Am J Transl Res 2021; 13: 10830-10836.

[9] Tao X, Austin RM, Yu T, Zhong F, Zhou X, Cong Q, Sui L and Zhao C. Risk stratification for cervical neoplasia using extended high-risk HPV genotyping in women with ASC-US cytology: a large retrospective study from China. Cancer Cytopathol 2022; 130: 248-258.

[10] Mbulawa ZZA, Phohlo K, Garcia-Jardon M, Williamson AL and Businge CB. High human papillomavirus (HPV)-35 prevalence among South African women with cervical intraepithelial neoplasia warrants attention. PLoS One 2022; 17: e0264498.

[11] Long W, Yang Z, Li X, Chen M, Liu J, Zhang Y and Sun X. HPV-16, HPV-58, and HPV-33 are the most carcinogenic HPV genotypes in Southwestern China and their viral loads are associated with severity of premalignant lesions in the cervix. Virol J 2018; 15: 94.

[12] Shanmugasundaram S and You J. Targeting persistent human papillomavirus infection. Viruses 2017; 9: 229.

[13] Koshiol J, Lindsay L, Pimenta JM, Poole C, Jenkins D and Smith JS. Persistent human papillomavirus infection and cervical neoplasia: a systematic review and meta-analysis. Am J Epidemiol 2008; 168: 123-137.

[14] Laganà AS, Chiantera V, Gerli S, Proietti S, Lepore E, Unfer V, Carugno J and Favilli A. Pre-

venting persistence of HPV infection with natural molecules. Pathogens 2023; 12: 416.
[15] WHO Guidelines Approved by the Guidelines Review Committee. WHO guideline for screening and treatment of cervical pre-cancer lesions for cervical cancer prevention. Geneva: World Health Organization© World Health Organization 2021; 2021.
[16] Papalia N, Rohla A, Tang S, Nation J and Nelson G. Defining the short-term disease recurrence after loop electrosurgical excision procedure (LEEP). BMC Womens Health 2020; 20: 34.
[17] Ding T, Li L, Duan R, Chen Y, Yang B and Xi M. Risk factors analysis of recurrent disease after treatment with a loop electrosurgical excision procedure for high-grade cervical intraepithelial neoplasia. Int J Gynaecol Obstet 2023; 160: 538-547.
[18] Gravitt PE and Winer RL. Natural history of HPV infection across the lifespan: role of viral latency. Viruses 2017; 9: 267.
[19] Della Fera AN, Warburton A, Coursey TL, Khurana S and McBride AA. Persistent human papillomavirus infection. Viruses 2021; 13: 321.
[20] Shimizu A, Yamaguchi R and Kuriyama Y. Recent advances in cutaneous HPV infection. J Dermatol 2023; 50: 290-298.
[21] Kuriyama Y, Kosaka M, Kaneko A, Nishioka H, Anzawa K, Hattori T, Igarashi N, Tamura M, Motegi SI and Shimizu A. Skin surface material for detecting human papillomavirus infection of skin warts. J Dermatol 2023; 50: 1450-1458.
[22] Cassani C, Dominoni M, Pasquali MF, Gardella B and Spinillo A. The role of multiple high-risk human papillomavirus infection on the persistence recurrence of high-grade cervical lesions after standard treatment: a systematic review and a meta-analysis. Acta Obstet Gynecol Scand 2024; 103: 1028-1035.
[23] Hebbar A and Murthy VS. Role of p16/INK4a and Ki-67 as specific biomarkers for cervical intraepithelial neoplasia: an institutional study. J Lab Physicians 2017; 9: 104-110.
[24] Mills AM. Biomarkers in cervical squamous neoplasia: diagnostic, prognostic, and predictive. Arch Pathol Lab Med 2025; 150: e46-e55.
[25] Stamatiou K, Huguet F, Serapinas LV, Spanos C, Rappsilber J and Vagnarelli P. Ki-67 is necessary during DNA replication for fork protection and genome stability. Genome Biol 2024; 25: 105.
[26] Muñoz N, Bosch FX, de Sanjosé S, Herrero R, Castellsagué X, Shah KV, Snijders PJ and Meijer CJ; International Agency for Research on Cancer Multicenter Cervical Cancer Study Group. Epidemiologic classification of human papillomavirus types associated with cervical cancer. N Engl J Med 2003; 348: 518-527.
[27] Kim M, Park NJ, Jeong JY and Park JY. Multiple human papilloma virus (HPV) infections are associated with HSIL and persistent HPV infection status in korean patients. Viruses 2021; 13: 1342.
[28] Dickson EL, Vogel RI, Bliss RL and Downs LS Jr. Multiple-type human papillomavirus (HPV) infections: a cross-sectional analysis of the prevalence of specific types in 309,000 women referred for HPV testing at the time of cervical cytology. Int J Gynecol Cancer 2013; 23: 1295-1302.
[29] Fife KH, Cramer HM, Schroeder JM and Brown DR. Detection of multiple human papillomavirus types in the lower genital tract correlates with cervical dysplasia. J Med Virol 2001; 64: 550-559.
[30] Herrero R, Castle PE, Schiffman M, Bratti MC, Hildesheim A, Morales J, Alfaro M, Sherman ME, Wacholder S, Chen S, Rodriguez AC and Burk RD. Epidemiologic profile of type-specific human papillomavirus infection and cervical neoplasia in Guanacaste, Costa Rica. J Infect Dis 2005; 191: 1796-1807.
[31] Adcock R, Cuzick J, Hunt WC, McDonald RM and Wheeler CM; New Mexico HPV Pap Registry Steering Committee. Role of HPV genotype, multiple infections, and viral load on the risk of high-grade cervical neoplasia. Cancer Epidemiol Biomarkers Prev 2019; 28: 1816-1824.
[32] Na J, Li Y, Wang J, Wang X, Lu J and Han S. The correlation between multiple HPV infections and the occurrence, development, and prognosis of cervical cancer. Front Microbiol 2023; 14: 1220522.
[33] Lili E, Chatzistamatiou K, Kalpaktsidou-Vakiani A, Moysiadis T and Agorastos T. Low recurrence rate of high-grade cervical intraepithelial neoplasia after successful excision and routine colposcopy during follow-up. Medicine (Baltimore) 2018; 97: e9719.
[34] Liu Y, Xu C, Pan J, Sun C, Zhou H and Meng Y. Significance of the viral load of high-risk HPV in the diagnosis and prediction of cervical lesions: a retrospective study. BMC Womens Health 2021; 21: 353.
[35] Malagón T, Louvanto K, Ramanakumar AV, Koushik A, Coutlée F and Franco EL; Biomarkers of Cervical Cancer Risk Study Team. Viral load of human papillomavirus types 16/18/31/33/45 as a predictor of cervical intraepithelial neoplasia and cancer by age. Gynecol Oncol 2019; 155: 245-253.
[36] Oyervides-Muñoz MA, Pérez-Maya AA, Sánchez-Domínguez CN, Berlanga-Garza A, Antonio-Macedo M, Valdéz-Chapa LD, Cerda-Flores RM, Trevino V, Barrera-Saldaña HA and Garza-

Rodríguez ML. Multiple HPV infections and viral load association in persistent cervical lesions in mexican women. Viruses 2020; 12: 380.

[37] Zhao X, Zhao S, Hu S, Zhao K, Zhang Q, Zhang X, Pan Q and Zhao F. Role of human papillomavirus DNA load in predicting the long-term risk of cervical cancer: a 15-year prospective cohort study in China. J Infect Dis 2019; 219: 215-222.

[38] Baumann A, Henriques J, Selmani Z, Meurisse A, Lepiller Q, Vernerey D, Valmary-Degano S, Paget-Bailly S, Riethmuller D, Ramanah R, Mougin C and Prétet JL. HPV16 load is a potential biomarker to predict risk of high-grade cervical lesions in high-risk HPV-infected women: a large longitudinal french hospital-based cohort study. Cancers (Basel) 2021; 13: 4149.

[39] Baser E, Ozgu E, Erkilinc S, Togrul C, Caglar M and Gungor T. Risk factors for human papillomavirus persistence among women undergoing cold-knife conization for treatment of high-grade cervical intraepithelial neoplasia. Int J Gynaecol Obstet 2014; 125: 275-278.

[40] Cai L, Huang Y, Lin C, Liu G, Mao X, Dong B, Lu T and Sun P. A comparison study of post-operative infection analysis of cold-knife conization and loop electrosurgical excision procedure for cervical high-grade squamous intraepithelial lesion. Transl Cancer Res 2020; 9: 949-957.

[41] Bogani G, Dl Donato V, Sopracordevole F, Ciavattini A, Ghelardi A, Lopez S, Simoncini T, Plotti F, Casarin J, Serati M, Pinelli C, Valenti G, Bergamini A, Gardella B, Dell'acqua A, Monti E, Vercellini P, Fischetti M, D'Ippolito G, Aguzzoli L, Mandato VD, Carunchio P, Carlinfante G, Giannella L, Scaffa C, Falcone F, Borghi C, Ditto A, Malzoni M, Giannini A, Salerno MG, Liberale V, Contino B, Donfrancesco C, Desiato M, Perrone AM, Dondi G, De Iaco P, Leone Roberti Maggiore U, Signorelli M, Chiappa V, Ferrero S, Sarpietro G, Matarazzo MG, Cianci A, Bosio S, Ruisi S, Guerrisi R, Brusadelli C, Mosca L, Tinelli R, DE Vincenzo R, Zannoni GF, Ferrandina G, Petrillo M, Dessole S, Angioli R, Greggi S, Spinillo A, Ghezzi F, Colacurci N, Muzii L, Benedetti Panici P, Scambia G and Raspagliesi F. Recurrence rate after loop electrosurgical excision procedure (LEEP) and laser Conization: a 5-year follow-up study. Gynecol Oncol 2020; 159: 636-641.

[42] Arbyn M, Redman CWE, Verdoodt F, Kyrgiou M, Tzafetas M, Ghaem-Maghami S, Petry KU, Leeson S, Bergeron C, Nieminen P, Gondry J, Reich O and Moss EL. Incomplete excision of cervical precancer as a predictor of treatment failure: a systematic review and meta-analysis. Lancet Oncol 2017; 18: 1665-1679.

[43] Zhang W and Lin Y. Modified method of cervical conization with hybrid use of a cold knife and an electric knife for high-grade squamous intraepithelial lesions. J Int Med Res 2022; 50: 3000605221106414.

[44] Lubrano A, Medina N, Benito V, Arencibia O, Falcón JM, Leon L, Molina J and Falcón O. Follow-up after LLETZ: a study of 682 cases of CIN 2-CIN 3 in a single institution. Eur J Obstet Gynecol Reprod Biol 2012; 161: 71-74.

[45] Kuroki LM, James-Nywening L, Wu N, Liu J, Powell MA, Thaker PH and Massad LS. High-grade cervical dysplasia after negative loop electrosurgical excision procedure. J Low Genit Tract Dis 2016; 20: 300-306.