Letter to the Editor

Obstetrics and Gynecology

# Analysing the factors related with persistent HPV positivity following LEEP surgery for cervical intraepithelial lesions

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**Submitted:** 15 October 2025; **Accepted:** 23 November 2025
**Online publication:** 18 December 2025

Arch Med Sci 2025; 21 (6): 2888–2890
DOI: https://doi.org/10.5114/aoms/214618


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Cervical intraepithelial neoplasia (CIN) is a critical precursor to cervical cancer, with persistent human papillomavirus (HPV) infection post-treatment being a key predictor of disease recurrence [1]. Loop electrosurgical excision procedure (LEEP) is a first-line treatment for CIN due to its efficacy and minimal invasiveness [2]. However, 20–30% of patients remain HPV-positive 1 year after surgery, significantly increasing the risk of recurrence and invasive cancer [3]. To contribute real-world insights, we analysed factors associated with persistent HPV positivity in 80 CIN patients post-LEEP.

We retrospectively enrolled 80 CIN patients with HPV infection who underwent LEEP between January 2020 and September 2022. The cohort included 18 CIN1 (22.5%), 35 CIN2 (43.75%), and 27 CIN3 (33.75%) cases. Inclusion criteria were as follows: histological confirmation of CIN, preoperative HPV positivity, no HPV vaccination history, and complete 1-year follow-up data (at 3, 6, and 12 months). Exclusion criteria included prior hysterectomy, concurrent malignancies, cervical cancer, pregnancy, severe comorbidities, or autoimmune diseases.

Preoperative evaluations included:

1) HPV testing: Using the Human Papillomavirus Genotyping Kit (manufactured by Da An Gene Co., Ltd., Guangzhou, China), we detected 15 high-risk (HR-HPV) types (HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, 73) and 2 low-risk (LR-HPV) types (HPV6, 11) via qPCR, with a detection limit of 500 copies/ml. Preoperative multiple HPV infections were defined as co-infection with 2 or more high-risk HPV types within the scope of this study; single low-risk HPV infection or mixed infection with a single HR-HPV were not included in this category.
2) Thinprep cytologic test (TCT): Following the Bethesda System 2014 criteria, results were classified as negative for intraepithelial lesion or malignancy (NILM), atypical squamous cells of undetermined significance (ASC-US), low-grade squamous intraepithelial lesion (LSIL), or high-grade squamous intraepithelial lesion (HSIL).
3) Vaginal microecology assessment: Vaginal secretions were tested for pH value, lactobacillus density (graded as I–IV), and the presence of bacterial vaginosis (BV) or vulvovaginal candidiasis (VVC).

LEEP was performed by 2 senior gynaecologists with > 5 years of LEEP experience. The surgical protocol was standardised: the excision margin was 3–5 mm beyond the visible lesion (confirmed by colposcopy

with acetic acid staining), and the excision depth was 1.5–2.5 cm (adjusted according to lesion location, e.g. 2.0–2.5 cm for endocervical lesions). Postoperatively, HPV testing was repeated at 3, 6, and 12 months; if any type of HPV was positive at the 12-month follow-up, it was defined as persistent infection. Clinical data were collected from electronic medical records, including age, parity, number of pregnancies, preoperative HPV genotyping results, TCT findings, vaginal microecology indicators, and postoperative pathological margin status (divided into [endocervical margin] and [exocervical margin] positivity). Positive postoperative resection margin was defined as follows: in postoperative pathological examination, when CIN cells were observed in the tissue sections of the cervical canal or external cervical orifice resection margins, it was determined as positive; if only HPV virus was detected but no CIN lesion cells were found, it was not considered a positive resection margin.

Statistical analysis was performed using SPSS 26.0: the $\chi^2$ test was used for categorical variables, the independent samples *t*-test was used for continuous variables, and multivariate logistic regression was used to identify independent risk factors (with variables with $p < 0.1$ in univariate analysis included in the regression model).

Among the 80 patients, 20 (25.00%) had persistent HPV positivity at 12 months postoperatively. Univariate analysis showed that the persistent group had a higher number of pregnancies (3.1 ±1.7 vs. 2.7 ±1.5, $t = 1.098$, $p = 0.045$), a higher rate of preoperative multiple HPV infections (55.0% vs. 25.0%, $\chi^2 = 7.280$, $p = 0.007$), and a higher rate of positive postoperative margins (45.0% vs. 13.3%, $\chi^2 = 10.536$, $p = 0.001$). Among the positive margins, 7 (77.8%) cases were endocervical margin positivity, and 2 (22.2%) cases were exocervical margin positivity. No significant differences were observed in age (42.3 ±6.8 vs. 40.5 ±7.2, $t = 1.023$, $p = 0.310$), hypertension (15.0% vs. 11.7%, $\chi^2 = 0.182$, $p = 0.670$), abnormal TCT (65.0% vs. 53.3%, $\chi^2 = 0.924$, $p = 0.336$), or vaginal microecology indicators (e.g. lactobacillus grade IV: 20.0% vs. 16.7%, $\chi^2 = 0.154$, $p = 0.695$; BV: 10.0% vs. 8.3%, $\chi^2 = 0.072$, $p = 0.788$) between the 2 groups (Table I). Multivariate logistic regression analysis (adjusted for age and number of pregnancies) confirmed that preoperative multiple HPV infection (OR = 4.213, 95% CI: 1.382–12.847, $p = 0.012$) and positive postoperative margins (OR = 5.876, 95% CI: 1.895–18.224, $p = 0.002$) were independent risk factors for persistent HPV positivity. The number of pregnancies, which was significant in univariate analysis, showed no statistical significance in multivariate analysis (OR = 1.205, 95% CI: 0.918–1.578, $p = 0.183$).

Our findings align with and extend previous research on HPV persistence post LEEP. The strong association between multiple HR-HPV infections and persistence is consistent with studies suggesting synergistic viral interactions that may overwhelm local immune clearance mechanisms and enhance viral oncoprotein activity, facilitating immune evasion and viral persistence [4]. Similarly, the significant risk posed by positive surgical margins, particularly endocervical margins, underscores the technical challenge of completely eradicating HPV-harbouring lesions from the cervical transformation zone, a factor well-documented in the literature [5]. Our results reinforce the critical importance of achieving clear margins during LEEP. The lack of significant association between vaginal microecology and HPV persistence in our cohort contrasts with some meta-analyses [6], which may be attributable to our relatively small sample size or differences in population characteristics.

The identified risk factors can be explained mechanistically:

Multiple HPV infection – co-infection with multiple high-risk HPV types may synergistically

**Table I.** Univariate analysis of factors for persistent HPV positivity after LEEP (*n*, %)

| Variable | Positive group (*n* = 20) | Negative group (*n* = 60) | $\chi^2$/*t* value | *P*-value |
|---|---|---|---|---|
| Age [years] x ± s | 42.3 ±6.8 | 40.5 ±7.2 | 1.023 | 0.310 |
| Pregnancies [times] x ± s | 3.1 ±1.7 | 2.7 ±1.5 | 1.098 | 0.045 |
| Preoperative multiple HPV infection | 11 (55.0) | 15 (25.0) | 7.280 | 0.007 |
| Postoperative margin positive | 9 (45.0) | 8 (13.3) | 10.536 | 0.001 |
| Endocervical margin positive | 7 (35.0) | 5 (8.3) | 8.944 | 0.003 |
| Exocervical margin positive | 2 (10.0) | 3 (5.0) | 0.556 | 0.456 |
| Abnormal TCT | 13 (65.0) | 32 (53.3) | 0.924 | 0.336 |
| ASC-US/LSIL | 8 (40.0) | 20 (33.3) | 0.286 | 0.593 |
| HSIL | 5 (25.0) | 12 (20.0) | 0.227 | 0.634 |
| Vaginal lactobacillus grade IV | 4 (20.0) | 10 (16.7) | 0.154 | 0.695 |
| Bacterial vaginosis (BV) | 2 (10.0) | 5 (8.3) | 0.072 | 0.788 |

enhance viral persistence through combined suppression of tumour suppressor pathways (e.g. p53 and Rb), facilitating immune evasion.

Positive Margins – residual infected cells, particularly at endocervical margins, are common due to limited colposcopic visualisation and surgical accessibility, directly contributing to persistence. The association between pregnancy number and HPV persistence in univariate analysis may reflect limited statistical power or confounding factors.

This study has several limitations. First, the single-centre retrospective design may introduce selection bias and limit the generalisability of the findings. Second, the small sample size ($n$ = 80) may reduce statistical power, making it difficult to detect risk factors with weak associations. Third, the follow-up period was limited to 1 year, and long-term outcomes were not evaluated. Fourth, potential confounders such as smoking and immune function were not included. Future multi-centre, prospective studies with larger sample sizes and longer follow-up periods are needed to validate these findings and explore additional influencing factors [7, 8].

Clinically, based on our findings, we recommend the following targeted measures: (1) Preoperative HPV genotyping to identify multiple high-risk infections, and consider endocervical curettage (ECC) to assess endocervical involvement. (2) Enhanced follow-up – for high-risk patients (multiple HPV infections or positive margins), shorten follow-up intervals to 3 months with combined HPV and TCT testing. (3) Patient education — emphasise protected intercourse, healthy lifestyle to boost immunity, and strict adherence to the follow-up scheme to mitigate persistence risks.

Preoperative multiple HPV infection and positive surgical margins are significant independent risk factors for persistent HPV positivity after LEEP. Targeted preoperative assessment and vigilant postoperative monitoring are essential for improving patient outcomes.

## Funding

No external funding.

## Ethical approval

Approval number: KYX2020049.

## Conflict of interest

The authors declare no conflict of interest.

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