

Impact of Post-LEEP Nonavalent HPV Vaccination on Follow-up Outcomes: A Single-Center Retrospective Cohort Study

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ABSTRACT

This study aimed to evaluate the effect of post-treatment administration of the nonavalent HPV vaccine on HPV clearance, cervical cytology outcomes, and HSIL recurrence during follow-up in women who underwent loop electrosurgical excision procedure (LEEP) for HSIL (histological HSIL).

This single-center retrospective cohort study included 200 women aged 18–65 years who underwent LEEP for histologically confirmed HSIL/CIN2–3 between 2020 and 2025 and had no prior HPV vaccination. Participants were grouped according to post-LEEP vaccination status: those who received the three-dose nonavalent HPV vaccine at 0, 2, and 6 months after LEEP (n=100) and unvaccinated controls (n=100). Co-testing was performed at 6 and 18 months, with colposcopy conducted as indicated per 2019 ASCCP guidelines.

There were no statistically meaningful differences between groups regarding HPV genotype distribution, or cytology findings. At 6 months, HPV negativity (70% vs. 77%; $p = 0.262$) and cytology negativity (73% vs. 76%; $p = 0.626$) rates were comparable between unvaccinated and vaccinated groups. At 18 months, the vaccinated group showed significantly higher HPV (91% vs. 81%; $p = 0.042$) and cytology negativity rates (93% vs. 81%; $p = 0.012$). HSIL rates were numerically lower in vaccinated patients at both follow-ups but did not reach statistical significance. Multivariate analysis showed HPV vaccination was independently associated with reduced 18-month HPV (OR = 0.42) and cytology positivity (OR = 0.32).

Post-LEEP nonavalent HPV vaccination was not associated with early follow-up differences but was independently linked to improved mid-term HPV clearance and cytologic outcomes.

Keywords: High-grade squamous intraepithelial lesion (HSIL), Loop electrosurgical excision procedure (LEEP), Nonavalent HPV vaccine, HPV clearance.

Introduction

Cervical cancer remains the fourth leading cancer diagnosis among women worldwide and continues to contribute substantially to the global health burden (1). Cervical intraepithelial neoplasia (CIN), a recognized precursor of cervical cancer, is highly associated with persistent infection caused by high-risk human papillomavirus (hrHPV) strains (2,3).

CIN is graded into three stages: CIN I represents mild epithelial dysplasia and is classified as low-grade squamous intraepithelial lesion (LSIL); CIN II reflects moderate dysplasia; and CIN III indicates severe

dysplasia. Both CIN II and CIN III are grouped under high-grade squamous intraepithelial lesions (HSIL) (4). Vaccination against hrHPV, together with early CIN detection and treatment through organized screening programs, forms the basis of effective cervical cancer prevention strategies (1,5). Therefore, many countries have integrated HPV vaccination into their national cervical cancer control and screening frameworks to reduce disease burden at the population level (6).

In women diagnosed with HSIL, excisional treatment is recommended to eradicate the precursor lesion and prevent progression to invasive cervical cancer (7).

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Although the effectiveness of excisional procedures has been well established, persistent or recurrent HSIL has been reported in approximately 5–15% of women undergoing cervical excision (7-9). Post-treatment HSIL may be associated with persistent HPV infection following excision, incomplete removal of the cervical lesion (treatment failure), or reinfection with a different HPV genotype (10-12). Moreover, despite adequate treatment, these women remain at a higher risk of developing cervical cancer compared with the general population and therefore require close surveillance due to the ongoing risk of post-treatment HSIL (13-15).

Several studies have suggested that both prophylactic and adjuvant HPV vaccination in women treated for HSIL may be associated with a reduced risk of persistent and/or recurrent HSIL (7,16,17). Antibody titers induced by HPV vaccination have been shown to be substantially higher than those generated following natural HPV infection (18,19). Moreover, it has been suggested that HPV vaccination may provide protective benefits even in women with existing HPV infection (14). However, evidence from randomized controlled trials in this setting remains limited.

The potential benefit of adjuvant vaccination may be explained by the enhanced immune response associated with increased antibody levels following immunization (20). According to the ASCCP guidelines, routine HPV vaccination is recommended for individuals aged 9–26 years. In unvaccinated women aged 27–45 years who have been treated for HSIL, the possible benefit of adjuvant HPV vaccination in reducing recurrence risk should be considered on an individual basis (21).

The present study aimed to evaluate the clinical outcomes associated with post-treatment administration of the nonavalent HPV vaccine in women who underwent LEEP for HSIL. The primary objective was to determine the association between adjuvant HPV vaccination and HPV clearance as well as cytologic and/or HPV test negativity during follow-up. The secondary objective was to investigate the potential role of vaccination in reducing the risk of persistent and/or recurrent HSIL.

Materials and Methods

The present study was structured as a retrospective observational cohort analysis and was performed at a single institution, between 2020 and 2025.

A total of 200 women aged 18–65 years who underwent excisional treatment with LEEP for histopathologically confirmed HSIL/CIN2–3 were

included. Patient selection was done by randomly choosing 100 patients at a time from the hospital's electronic information system.

Patients with no prior history of HPV vaccination before diagnosis were categorized into two groups according to their post-LEEP vaccination status. The vaccinated group (n=100) consisted of patients whose vaccination records were verified through the hospital electronic medical record system and who completed the full three-dose schedule of the nonavalent HPV vaccine administered at 0, 2, and 6 months following LEEP.

Patients with ongoing pregnancy, those diagnosed with multicentric HPV-related disease, and those with a prior history of excisional treatment for cervical lesions were excluded.

In addition, women diagnosed with invasive cervical cancer on histopathological evaluation of the excisional specimen, those with a history of hysterectomy, and those with missing or unverifiable HPV vaccination data were excluded from the analysis.

Approval for this research was obtained from our Local Research Ethics Committee. (Approval Number: 720; Date: 7.11.2025). All study procedures were carried out in compliance with the ethical guidelines of the Declaration of Helsinki.

In this retrospective review, demographic and clinical characteristics were collected using the hospital's electronic medical records. The dataset included HPV vaccination status, age, body mass index (BMI), gravidity, parity, smoking status, contraceptive method, cervical cytology at presentation, baseline HPV test results, and HPV genotype distribution.

In addition, colposcopy-directed cervical biopsy results, depth of the LEEP specimen (in centimeters), histopathological diagnosis, and surgical margin status were recorded.

Cervical cytology samples were collected in accordance with standard clinical practice. After a speculum was placed in the lithotomy position, cervical mucus was gently removed, and samples were obtained from both the ectocervix and endocervix using a cervical cytobrush (Medbar Medical, Turkey).

The collected specimens were transferred into tubes containing Path Store® Cell Preservative Solution and processed using the liquid-based cytology (LBC) method. Cytologic evaluation was performed and reported according to The Bethesda System for Reporting Cervical Cytology (TBS) criteria (22).

HPV samples were collected during the same visit using the Abbott Cervi-Collect® specimen collection

device (Abbott Laboratories, USA) and transferred into Abbott Cervi-Collect Preservative Medium.

High-risk HPV (hrHPV) DNA analysis was performed using the Abbott RealTime High Risk HPV assay in accordance with the manufacturer's instructions. The assay separately identifies HPV-16 and HPV-18 genotypes, while the remaining 12 high-risk HPV genotypes are reported collectively as "other high-risk HPV."

Indications for colposcopy were determined according to the 2019 ASCCP risk-based management guidelines. Colposcopy was performed in patients with an immediate risk of CIN3+ of $\geq 4\%$ (23).

Colposcopic evaluation was performed in accordance with the 2019 ASCCP guidelines and international standards. Following the application of 3–5% acetic acid solution, the cervix was examined under colposcopic visualization, with careful assessment of acetowhite changes, vascular patterns, and lesion margins.

Targeted biopsies were obtained from any suspicious areas identified during colposcopy, and endocervical curettage was performed when indicated. In patients with positive hrHPV results but no visible colposcopic lesions, at least two random biopsies were obtained from different cervical quadrants to increase diagnostic sensitivity (23).

Cervical biopsy and endocervical curettage specimens obtained under colposcopic guidance were fixed in formalin and embedded in paraffin. Sections of 4 μm thickness were stained with hematoxylin and eosin (H&E), and p16 immunohistochemical staining was performed when indicated.

Histopathological diagnoses were stratified into negative, LSIL/CIN1, HSIL/CIN2, and HSIL/CIN3 categories in accordance with the WHO criteria and the LAST terminology framework (24,25).

Management of HSIL cases was planned in accordance with the 2019 ASCCP guidelines. In patients diagnosed with CIN2, the decision to perform excisional treatment was individualized based on reproductive intentions. In contrast, all patients diagnosed with CIN3 underwent LEEP regardless of fertility considerations (23).

LEEP was performed in all patients under sedation with local anesthesia in the dorsal lithotomy position. After adequate local anesthesia of the cervix was achieved according to standard clinical practice, an appropriately sized electrosurgical loop was selected, and excision was performed to include the transformation zone.

The excised cervical specimen was properly oriented and sent for histopathological evaluation. Hemostasis was achieved using electrocauterization when necessary.

Surgical specimens obtained by LEEP were classified as negative for SIL, LSIL, or HSIL according to the WHO criteria (26). Surgical margins were inked and evaluated histopathologically. Cases with no evidence of SIL at the resection margins were considered margin-negative, whereas the presence of SIL at any surgical margin was defined as margin-positive.

In patients with positive surgical margins, treatment options—including repeat LEEP (re-LEEP) or hysterectomy—were offered based on current guideline recommendations and individual patient characteristics, such as age, reproductive intentions, and accompanying risk factors.

Patients who underwent LEEP for the treatment of HSIL were advised to receive the nonavalent HPV vaccine as adjuvant therapy according to the standard three-dose schedule administered at 0, 2, and 6 months (27).

All patients underwent co-testing (HPV testing and cervical cytology) at the first follow-up visit scheduled 6 months after treatment, in accordance with the 2019 ASCCP risk-based management guidelines recommending HPV-based testing after excisional treatment for HSIL. If both HPV and cytology results were negative at this visit, a second follow-up was planned 12 months later (18 months after treatment), at which time co-testing was repeated.

In the analyses of follow-up visits, colposcopic findings were evaluated only among patients who met the indication criteria for colposcopy according to the 2019 ASCCP guidelines.

Cases in which LSIL or HSIL was identified on colposcopy-directed biopsy were classified as having a positive colposcopic result, whereas those with no pathological findings on biopsy were classified as negative.

Patients diagnosed with HSIL on colposcopy-directed cervical biopsy and/or endocervical curettage at any follow-up visit were referred for repeat LEEP.

The primary endpoint of this study was to evaluate the effect of post-treatment nonavalent HPV vaccination on HPV clearance in patients who underwent LEEP for HSIL. HPV clearance was defined as negativity on cervical cytology and HPV testing during follow-up.

The secondary endpoint was the comparison of persistent and/or recurrent HSIL rates between vaccinated and unvaccinated patients.

Statistical Analysis: Data analysis was performed using IBM SPSS Statistics version 26.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics included frequencies (n) and percentages (%) for categorical data, and mean $\pm$ standard deviation (SD) values for continuous measurements.

Comparisons between vaccinated and unvaccinated groups were conducted using the chi-square (χ^2) test for categorical variables, including smoking status, contraceptive method, cytology findings, colposcopy results, histopathological diagnoses, smear results, and HPV genotype distribution. When expected cell counts were insufficient, appropriate corrections were applied.

Continuous variables, including age, gravidity, parity, and depth of the LEEP specimen, were compared between groups using the independent samples t-test.

Logistic regression analysis was performed to evaluate factors associated with HPV vaccination status. In this model, vaccination status was entered as the dependent variable, while 18-month HPV test results and 18-month smear results were included as independent variables.

Regression findings were reported in terms of odds ratios (ORs) and 95% confidence intervals (CIs). The model's explanatory performance was measured using the Nagelkerke R^2 coefficient. For all analyses, a p-value below 0.05 was considered statistically significant. Categorical outcome variables were compared between the vaccinated and unvaccinated groups using Pearson's chi-square test of independence.

Power analysis to determine sample size adequacy was conducted using G*Power software (version 3.1.9.7) based on a 2×2 contingency table model. The analysis assumed a medium effect size (Cohen's $w = 0.20$), a significance threshold of $\alpha = 0.05$, and a target statistical power of $(1 - \beta) = 0.80$.

The calculated minimum sample size requirement was 197 participants. Given that 200 participants were included in this study, the achieved statistical power exceeded 0.80, demonstrating that the sample size was statistically sufficient.

Results

As presented in Table 1, the vaccinated and unvaccinated groups were comparable with respect to age (41.96 ± 8.81 vs. 40.87 ± 8.80 years; $p = 0.382$), gravidity (3.49 ± 2.03 vs. 3.58 ± 1.91 ; $p = 0.747$), parity (3.35 ± 2.00 vs. 3.50 ± 1.88 ; $p = 0.585$), and pathological depth of the LEEP specimen (2.04 ± 0.62 vs. 1.94 ± 0.64 cm; $p = 0.231$). Smoking status (43.8% vs.

56.2%; $p = 0.118$) and distribution of contraceptive methods ($p = 0.595$) were also similar between groups. The distribution of smoking status and contraceptive methods was similar between the two groups ($p = 0.118$ and $p = 0.595$, respectively). Likewise, no statistically significant differences were observed between the groups regarding colposcopic biopsy results ($p = 0.716$) or positive surgical margin rates following LEEP (13% vs. 15%; $p = 0.684$).

Table 2 presents the distribution of baseline HPV genotype, cervical cytology findings, and LEEP histopathological results, as well as HPV, smear, and colposcopic evaluation outcomes at the 6- and 18-month follow-up visits in the vaccinated and unvaccinated groups. There were no statistically significant differences between the groups regarding baseline HPV genotype distribution or cervical cytology results ($p = 0.630$ and $p = 0.329$, respectively). However, a significant difference was found in the histopathological evaluation of the LEEP specimens, with a higher rate of negative histology in the unvaccinated group compared to the vaccinated group (8% vs. 1%, $p = 0.017$).

At the 6-month follow-up, no statistically significant differences were observed between the groups in terms of HPV negativity (70% vs. 77%) or smear negativity rates (73% vs. 76%) (all $p > 0.05$). Colposcopic evaluation at 6 months was performed only when indicated. Colposcopic evaluation was carried out in 29 patients from the unvaccinated cohort and 23 patients from the vaccinated cohort. In the unvaccinated group, 21 of 29 patients (72.4%) had positive colposcopy-directed biopsy findings (LSIL: 15; HSIL: 6), whereas 8 patients (27.6%) had negative biopsy results. In the vaccinated group, positive colposcopic findings were observed in 13 of 23 patients (56.5%) (LSIL: 8; HSIL: 5), while 10 patients (43.5%) had negative biopsy results.

No significant difference in colposcopic results was found between the groups at the 6-month follow-up ($p = 0.232$). However, at 18 months, the vaccinated group demonstrated significantly higher rates of HPV negativity (91% vs. 81%; $p = 0.042$) and smear negativity (93% vs. 81%; $p = 0.012$) compared with the unvaccinated group. Patients meeting the indication criteria for colposcopy at 18 months were re-evaluated. In the unvaccinated group, 20 patients underwent colposcopy, of whom 12 (60%) had positive colposcopy-directed biopsy findings (LSIL: 7; HSIL: 5), while 8 patients (40%) had negative biopsy results.

In the vaccinated group, 9 patients underwent colposcopy. Positive colposcopy-directed biopsy findings were identified in 7 patients (77.8%) (LSIL: 5; HSIL: 2), whereas no pathological findings were

Table 1: Baseline Demographic and Clinical Characteristics of the Study Groups

Variable	Unvaccinated	Vaccinated	P
Age (years)	40.87 ± 8.80	41.96 ± 8.81	0.382
Gravidity Parity	3.58±1.91	3.49±2.03	0.747
LEEP specimen depth (cm)	3.5±1.88	3.35±2	0.585
Smoking status	1.94±0.64	2.04±0.62	0.231
Non-smoker	50(45)	61(55)	0.118
Smoker	50(56.2)	39(43.8)	
Contraceptive method			
No method			0.595
Condom	16(51.6)	15(48.4)	
IUD	13(41.9)	18(58.1)	
Oral contraceptive			0.595
Colposcopic biopsy result			
CIN2	53(54.1)	45(45.9)	
CIN3	18(45)	22(55)	0.716
CIN2+CIN3			
LEEP surgical margin status			
Negative	20(52.6)	18(47.4)	0.716
Positive	67(50.8)	65(49.2)	
	13(43.3)	17(56.7)	
	87(50.6)	85(49.4)	0.684
	13(46.4)	15(53.6)	

* $p < 0.05$ was considered statistically significant. Continuous variables are presented as mean ± standard deviation (SD) and were compared using the independent samples t-test. Categorical variables are expressed as number (n) and percentage (%) and were analyzed using the chi-square (χ^2) test. CIN: Cervical intraepithelial neoplasia; LEEP: Loop electrosurgical excision procedure; OCP: Oral contraceptive pill; IUD: Intrauterine device; cm, centimeter

observed in 2 patients (22.2%). There was no statistically significant difference between the groups regarding colposcopic evaluation outcomes at the 18-month follow-up ($p = 0.351$).

When calculated based on the initial cohort ($n = 100$ per group), HSIL rates at the 6-month follow-up were 6% in the unvaccinated group and 5% in the vaccinated group, with no statistically significant difference between groups.

Using the same approach, HSIL rates at the 18-month follow-up were 5% in the unvaccinated group and 2% in the vaccinated group. Although the rate was numerically lower in the vaccinated group, this difference did not reach statistical significance ($p > 0.05$).

In the multivariable logistic regression analysis presented in Table 3, factors potentially associated with 18-month HPV positivity were evaluated. Age, smoking status, HPV vaccination status, surgical margin status of the LEEP specimen, and baseline cervical smear status were included in the model. The analysis demonstrated that HPV vaccination status was independently associated with 18-month HPV positivity. Vaccinated patients had a significantly lower likelihood of HPV positivity at 18 months

compared with unvaccinated patients (OR = 0.42; 95% CI: 0.18–0.98; $p = 0.046$). In contrast, age, smoking status, positive surgical margins, and baseline smear positivity were not significantly associated with 18-month HPV positivity (all $p > 0.05$).

In the multivariable logistic regression analysis presented in Table 4, factors potentially associated with 18-month smear positivity were examined. Age, smoking status, HPV vaccination status, surgical margin status of the LEEP specimen, and baseline smear status were included in the model. The analysis demonstrated that HPV vaccination status was independently associated with 18-month smear positivity. Vaccinated patients had a significantly lower likelihood of smear positivity at 18 months compared with unvaccinated patients (OR = 0.32; 95% CI: 0.13–0.80; $p = 0.015$). In contrast, age, smoking status, positive surgical margins, and baseline smear positivity were not significantly associated with 18-month smear positivity (all $p > 0.05$).

Discussion

In this single-center retrospective cohort study, we evaluated the impact of post-treatment administration

Table 2: Distribution of HPV, Cytology, and Colposcopic Biopsy Findings at Baseline and During Follow-up (6 and 18 Months) According to Vaccination Status

Variable	Groups	Unvaccinated n:100	Vaccinated n:100	p
Baseline HPV genotype	Other	34(34.0)	39(39.0)	0.630
	hrHPV+			
	HPV16	42(42.0)	42(42.0)	
Baseline cytology	HPV18	24(24.0)	19(19.0)	0.329
		13(13.0)	18(18.0)	
	Negative			
LEEP histopathology	Positive	87(87.0)	82(82.0)	0.017 *
		8(8.0)	1(1.0)	
	Negative			
6-month HPV result	Positive	92(92.0)	99(99.0)	0.262
		70(70.0)	77(77.0)	
	Negative			
6-month cytology	Positive	30(30.0)	23(23.0)	0.626
		73(73.0)	76(76.0)	
	Negative			
6-month colposcopy result	Positive	27(27.0)	24(24.0)	0.232
		8(27.6)	10(43.5)	
	Negative			
18-month HPV result	Positive	21(72.4)	13(56.5)	0.042 *
		81(81.0)	91(91.0)	
	Negative			
18-month cytology	Positive	19(19.0)	9(9.0)	0.012 *
		81(81.0)	93(93.0)	
	Negative			
18-month colposcopy result	Positive	19(19.0)	7(7.0)	0.351
		8(40.0)	2(22.2)	
	Negative			
	Positive	12(60.0)	7(77.8)	

* $p < 0.05$ was considered statistically significant. Data are presented as n (%). Comparisons between groups for categorical variables were performed using the chi-square (χ^2) test. Percentages for colposcopy results were calculated only among patients who underwent colposcopy (n/N_colposcopy). HPV: Human papillomavirus; LEEP: Loop electrosurgical excision procedure; CIN: Cervical intraepithelial neoplasia; Smear: Cervical cytology

of the nonavalent HPV vaccine on follow-up outcomes in women who underwent LEEP for HSIL/CIN2–3. Furthermore, there were no statistically significant differences between the groups in the distribution of CIN2–CIN3 on colposcopic biopsy ($p = 0.716$) or in the rates of positive surgical margins following LEEP (13% vs. 15%; $p = 0.684$). This comparability suggests that the groups were homogeneous prior to treatment and supports the interpretation that the differences observed in HPV clearance and HSIL recurrence during follow-up are more likely attributable to the potential effects of adjuvant HPV vaccination rather than to established prognostic factors such as age, surgical margin status, or lesion depth (28–31).

The absence of significant differences between the groups in baseline HPV genotype distribution and cervical cytology results ($p = 0.630$ and $p = 0.329$, respectively) further indicates that the two groups were comparable in terms of pre-treatment virological and cytological risk profiles. Consistent with the literature, baseline equivalence in these variables strengthens internal validity and allows differences observed during follow-up, particularly in HPV clearance and recurrence rates, to be more confidently attributed to post-treatment factors such as adjuvant HPV vaccination rather than to pre-existing disparities between groups (1,16, 32–34,35).

Excision of the HPV-related primary lesion may modulate the local inflammatory response through alterations in the cervical cytokine profile. In patients

Table 3: Multivariate Logistic Regression Analysis for 18-Month HPV Positivity

Independent variables	B	SE	P	Odds Ratio	95%C.I.for EXP(B)	
					Lower	Upper
Age	- 0.029	0.035	0.403	0.971	0.907	1.040
Smoking status (smoker)	0.612	0.441	0.165	1.845	0.778	4.375
HPV vaccination status (vaccinated)	- 0.864	0.433	0.046	0.422	0.181	0.984
LEEP surgical margin (positive)	- 0.905	0.787	0.250	0.404	0.086	1.893
Baseline cytology (positive)	0.018	0.614	0.977	1.018	0.306	3.391

* $p < 0.05$ was considered statistically significant. B: Regression coefficient; SE: Standard error; OR, Odds ratio; 95% CI, 95% confidence interval. Dependent variable: 18-month HPV positivity. HPV: Human papillomavirus; LEEP: Loop electrosurgical excision procedure

Table 4: Multivariate Logistic Regression Analysis for 18-Month Cytology Positivity

Independent variables	B	SE	P	Odds Ratio	95%C.I.for EXP(B)	
					Lower	Upper
Age	-0.010	0.035	0.776	0.990	0.924	1.061
Smoking (smoker)	0.586	0.454	0.198	1.796	0.737	4.376
HPV vaccination status (vaccinated)	-1.137	0.468	0.015	0.321	0.128	0.802
LEEP surgical margin (positive)	-0.817	0.795	0.304	0.442	0.093	2.098
Baseline cytology (positive)	0.371	0.694	0.593	1.449	0.372	5.643

* $p < 0.05$ was considered statistically significant. Dependent variable: 18-month cytology result. Regression coefficient; SE, Standard error; OR, Odds ratio; 95% CI, 95% confidence interval. HPV: Human papillomavirus LEEP: Loop electrosurgical excision procedure

undergoing LEEP, a significant early postoperative reduction in $\text{TNF-}\alpha$ levels has been reported, suggesting a shift in the local immune microenvironment following removal of the lesion. This finding suggests that removal of the primary lesion may reconstruct the cervical mucosal immune environment in a manner similar to the profile observed in individuals unexposed to HPV [36]. We believe that the increase in HPV and cytology negativity rates observed at the 6-month follow-up in both groups may be attributable primarily to the reduction in viral load achieved through surgical excision and the subsequent activation of host immune clearance mechanisms, rather than to an early effect of HPV vaccination.

In contrast, the findings at the 18-month follow-up represent the most notable results of our study. At 18 months, the HPV negativity rate was 91% in the

vaccinated group compared with 81% in the unvaccinated group, and this difference reached statistical significance ($p = 0.042$).

Consistent with these findings, Chen et al. examined the role of adjuvant HPV vaccination following LEEP in patients managed for HSIL and demonstrated that HPV infection rates at the second-year follow-up were significantly greater among unvaccinated patients than vaccinated patients (37).

In the study by Marie et al., consistent with our findings, no significant difference in HPV positivity rates was observed between vaccinated and unvaccinated groups at the 6-month follow-up. However, after two years of follow-up, a marked reduction in HPV positivity was reported in the vaccinated group, with HPV negativity rates of 91% and 84.6% in the vaccinated and unvaccinated groups (34,38).

According to current evidence, postoperative HPV vaccination in previously unvaccinated patients may confer protection against new HPV infections (14). Conversely, in women in whom HPV reactivation or reinfection occurs and long-term immune protection may be insufficient, HPV vaccination could potentially mitigate waning immune responses. In unvaccinated individuals, this immunological vulnerability may contribute to an increased risk of HPV-related recurrence.

The possible protective effect of postoperative HPV vaccination against viral reactivation and reinfection is further reinforced by immunological data showing that persistent HPV infection is linked to local microenvironmental changes, including increased levels of proinflammatory cytokines (33).

Moreover, our findings indicate that while the effect of HPV vaccination appears limited in the early postoperative period, its impact becomes more pronounced beyond six months, contributing significantly to mid-term viral clearance and cytological improvement.

Recent studies have reported a consistent adjuvant benefit of post-conization HPV vaccination, ranging between 58% and 80%, regardless of vaccine type (including 9vHPV) or timing of administration relative to excision (7,16,30,39).

In our study, colposcopy-directed biopsy positivity rates at both the 6- and 18-month follow-up visits did not differ significantly between the vaccinated and unvaccinated groups when analyses were restricted to patients undergoing colposcopy based on indication. However, when calculated based on the initial cohort, HSIL rates were consistently lower in the vaccinated group at both follow-up time points. At 18 months, HSIL rates were 5% in the unvaccinated group and 2% in the vaccinated group. Nevertheless, this trend is consistent with findings reported in the literature and may suggest that adjuvant HPV vaccination is associated with lower HSIL rates in vaccinated patients, even when statistical significance is not achieved. This trend, in line with previously published data, may indicate a potential association between adjuvant HPV vaccination and lower HSIL rates in the vaccinated group, despite the lack of statistical significance.

Multivariable analyses in our study demonstrated that adjuvant HPV vaccination provided an independent and statistically significant protective effect against both HPV positivity and smear positivity at the 18-month follow-up. Among vaccinated patients, the risk of HPV positivity at 18 months was significantly reduced (OR=0.42; 95% CI: 0.18–0.98), and a comparable reduction was observed in the likelihood

of smear positivity (OR=0.32; 95% CI: 0.13–0.80). In contrast, age, smoking status, LEEP surgical margin status, and baseline cytology findings were not independently associated with HPV or smear positivity at 18 months. These findings indicate that, by controlling for potential confounding factors through multivariable modeling, our study more clearly demonstrates the intermediate-term effect of adjuvant HPV vaccination. The clinically meaningful and statistically consistent results strengthen the methodological robustness of our study and highlight the independent contribution of HPV vaccination in the post-treatment follow-up setting.

A major strength of this study is that the effect of postoperative nonavalent HPV vaccination in women undergoing LEEP for HSIL was assessed between two groups that were comparable with respect to baseline demographic, clinical, and pathological characteristics. The absence of significant differences between the vaccinated and unvaccinated groups in terms of age, obstetric characteristics, smoking status, baseline HPV genotypes, cytological findings, lesion depth, and surgical margin status suggests that the differences observed during follow-up are more likely attributable to adjuvant HPV vaccination rather than to established prognostic factors. Furthermore, adjustment for potential confounders through multivariable analyses enhances the robustness and reliability of our findings.

Key limitations of this study include its retrospective, single-center, and non-randomized design, which may have introduced selection bias. As vaccination status was determined by patients' post-treatment decisions rather than random allocation, unmeasured confounding factors may have influenced the observed outcomes. Nevertheless, the use of a standardized treatment and follow-up protocol enhances the clinical relevance of our findings.

This study demonstrates that postoperative administration of the nonavalent HPV vaccine in women undergoing LEEP for HSIL/CIN2–3 is independently and significantly associated with improved mid-term HPV clearance and cytological remission. While no significant benefit was observed in the early follow-up period, the protective effect became more apparent over time. These findings support the potential role of adjuvant HPV vaccination in the post-treatment surveillance of women treated for HSIL.

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