Original Article

DOI: 10.4274/anajog.galenos.2026.70783

Anat J Obstet Gynecol Res 2026;3(2):112-117

Adjuvant Nonavalent HPV Vaccination is Associated with Improved One-Year HPV Clearance and Cervical Cytological Outcomes in Women with Histopathologically Confirmed LSIL/CIN1: A Retrospective Comparative Cohort Study

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ABSTRACT

Purpose: To compare one-year human papilloma virus (HPV) deoxyribonucleic acid and cervical cytology outcomes between women with histopathologically confirmed low-grade squamous intraepithelial lesions/cervical intraepithelial neoplasia grade 1 (LSIL/CIN1) who received adjuvant nonavalent HPV vaccination and a contemporaneous unvaccinated control cohort, and to identify baseline predictors of persistent disease.

Methods: This single-center retrospective comparative cohort study included women with histopathologically confirmed LSIL/CIN1, divided into vaccinated and unvaccinated (control) subgroups, who were treated between 2020 and 2025. The primary outcomes were one-year HPV clearance and cytological normalization. Complete regression was defined as concurrent HPV negativity and normal cervical cytology, whereas persistent disease was defined as persistent HPV positivity together with abnormal cervical cytology. Univariable logistic regression analysis was performed to identify predictors of persistent disease in the vaccinated cohort.

Results: Of the study cohort of 233 women, 153 (65.7%) women completed the three-dose nonavalent HPV vaccination schedule, while 80 (34.3%) women served as controls. Baseline demographic and clinical characteristics were comparable between the groups. At 12 months, HPV clearance was significantly more prevalent in the vaccinated group than in the control group (70.6% vs. 52.5%, $p=0.009$). Cytological normalization was also more frequent in vaccinated women (72.5% vs. 26.2%, $p<0.001$). Complete regression occurred in 53.6% of vaccinated women compared with 16.2% of controls ($p<0.001$), and consequently persistent disease was significantly less common in the vaccinated group (10.5% vs. 37.5%, $p<0.001$). Although HPV16, HPV18, other high-risk HPV types, and multiple HPV infections decreased in both groups, genotype-specific differences were not statistically significant. Baseline HPV18 positivity was the only significant predictor of persistent disease (odds ratio: 3.97, 95% confidence interval: 1.29-12.24; $p=0.017$).

Conclusion: Adjuvant nonavalent HPV vaccination was associated with higher HPV clearance, improved cervical cytological outcomes, higher complete regression, and lower persistent disease rates in women with histopathologically confirmed LSIL/CIN1. Prospective multicenter studies are warranted to confirm these findings and further define the role of adjuvant HPV vaccination in the conservative management of LSIL/CIN1.

Keywords: LSIL/CIN1, human papillomavirus, nonavalent HPV vaccine, HPV clearance, cervical cytology, persistent disease

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Received: 23.05.2026 **Accepted:** 08.07.2026 **Publication Date:** 02.09.2026

Cite this article as: Halisçelik MA, Oğlak SC, Şeker E, Yeleç S, Aydın MF, Kavak SB. Adjuvant nonavalent HPV vaccination is associated with improved one-year HPV clearance and cervical cytological outcomes in women with histopathologically confirmed LSIL/CIN1: a retrospective comparative cohort study. Anat J Obstet Gynecol Res. 2026;3(2):112-117

INTRODUCTION

Although cervical cancer is largely preventable, it continues to pose a major global health burden and remains one of the most frequently diagnosed malignancies in women worldwide.[1,2] Persistent infection with high-risk human papilloma virus (HPV) is the main etiological contributor to cervical carcinogenesis, with HPV-16 and HPV-18 genotypes responsible for approximately 70% of cases.[2,3] Persistent high-risk HPV infection may lead to the development of cervical intraepithelial neoplasia (CIN), which can progress from CIN1 to CIN2 and CIN3 and, if left untreated, eventually to invasive cervical cancer.[4] low-grade squamous intraepithelial lesions (LSIL) or CIN1 typically represent the early phase of HPV infection and, although a substantial proportion of these lesions undergo spontaneous regression, some cases may progress to persistent infection and disease progression.[4,5]

Prophylactic vaccines developed against HPV play a crucial role in the prevention of cervical cancer, and bivalent, quadrivalent, and nonavalent HPV vaccines are currently in clinical use.[1,6] The nonavalent HPV vaccine provides broader protection against oncogenic HPV types by covering HPV-6, HPV-11, HPV-16, HPV-18, as well as HPV-31, HPV-33, HPV-45, HPV-52, and HPV-58.[6] In recent years, evidence has emerged suggesting that HPV vaccination may not only serve a primary preventive role but also enhance viral clearance in HPV-positive women and reduce the risk of persistence or recurrence of HPV-related lesions. However, evidence regarding the adjuvant use of HPV vaccination in women with histologically confirmed LSIL/CIN1 remains limited, particularly from comparative studies including unvaccinated control groups.[7,8]

The aim of this retrospective comparative cohort study was to compare one-year HPV deoxyribonucleic acid (DNA) and cervical cytology outcomes between women with histopathologically confirmed LSIL/CIN1 who received adjuvant nonavalent HPV vaccination and a contemporaneous unvaccinated control cohort. Secondary objectives were to evaluate complete regression, persistent disease, and baseline clinical factors associated with persistent disease.

METHODS

This single-center retrospective comparative cohort study was conducted at the Department of Obstetrics and Gynecology, University of Health Sciences Turkey, Diyarbakır Gazi Yaşargil Research and Training Hospital, between 2020 and 2025. Women aged 18-65 years with histopathologically confirmed LSIL/CIN1 and no previous history of HPV vaccination were eligible for inclusion.

Patients in the vaccination group received the nonavalent HPV vaccine (Gardasil 9®, Merck Sharp & Dohme LLC, Rahway, NJ, USA) according to the standard three-dose schedule (0, 2, and 6 months). The control cohort included contemporaneous women diagnosed during the same study period who met the same inclusion and exclusion criteria but did not receive HPV vaccination.

Patients who were pregnant or postpartum, receiving immunosuppressive therapy, previously vaccinated against HPV, had undergone prior cervical excisional treatment, were diagnosed with HSIL/CIN2-3 or invasive cervical cancer, or had incomplete clinical follow-up were excluded from cohorts.

Ethical approval for the study was obtained from the Clinical Research Ethics Committee of University of Health Sciences Turkey, Diyarbakır Gazi Yaşargil Training and Research Hospital (approval no: 737, date: 21.11.2025), and the study was conducted in accordance with the principles of the Declaration of Helsinki.

Demographic and clinical data were obtained from the hospital's electronic medical record system. These included age, body mass index (BMI), gravida, parity, smoking status, contraceptive method, baseline cytology and HPV test results, HPV genotype, and histopathological findings from colposcopy-guided biopsies.

Cervical cytology samples were collected under standard conditions, processed using liquid-based cytology, and evaluated according to the Bethesda system.[9] HPV samples were collected during the same visit and analyzed using the Abbout, İstanbul, Turkey real time high-risk HPV assay. HPV-16 and HPV-18 genotypes were reported separately, while other high-risk HPV types were reported collectively.

Indications for colposcopy were determined according to the 2019 risk-based guidelines of the American Society for Colposcopy and Cervical Pathology.[10] All patients included in the study underwent colposcopy-guided cervical biopsy before enrollment, and only women with histopathologically confirmed LSIL/CIN1 were included in the final analysis. Endocervical curettage was performed when clinically indicated.[10]

Histopathological evaluation was performed using hematoxylin-eosin staining, and p16 immunohistochemistry was applied when necessary. Diagnoses were classified according to the World Health Organization and Lower Anogenital Squamous Terminology criteria.[11,12]

Colposcopies were performed by a second-year gynecological oncology fellow or a gynecologist with three years of experience.

Adjuvant nonavalent HPV vaccination was offered to patients diagnosed with LSIL/CIN1.[5] The primary outcomes were one-year HPV clearance and regression of cytological abnormalitiesin the vaccinated and unvaccinated cohorts. Complete regression was defined as the presence of both a negative HPV test and normal cervical cytology at the 12-month follow-up. Persistent disease was defined as persistent HPV positivity together with abnormal cervical cytology at the same follow-up visit. These outcome measures were used to compare the clinical outcomes between the two cohorts.

Statistical Analysis

Statistical analyses were performed using SPSS, version 22.0 (IBM Corp., Armonk, NY, USA). Continuous variables are expressed as mean ± standard deviation, while categorical variables are presented as frequencies and percentages.

Comparisons between groups were performed using the independent samples t-test for continuous variables. Categorical variables were compared using the chi-square test, and Fisher's exact test was applied when the expected cell count was less than five.

Changes in HPV status and cervical cytology between baseline and the 12-month follow-up were evaluated both within and between groups using these methods. Differences between groups in the primary outcomes, including HPV clearance, cytological normalization, complete regression, and persistent disease, were assessed using the chi-square test.

Univariable logistic regression analysis was performed in the vaccinated cohort to identify potential predictors of persistent disease. Age, BMI, gravida, parity, smoking status, and baseline HPV18 positivity were included in the model. The results are reported as odds ratios (ORs) with 95% confidence intervals (95% CIs). However, because the number of events per variable (EPV=2.7) was relatively low, the regression findings were interpreted as exploratory. A two-sided p value of <0.05 was considered statistically significant for all analyses.

RESULTS

A total of 233 women with histopathologically confirmed LSIL/CIN1 were included in the study, comprising 153 (65.7%) women who received adjuvant nonavalent HPV vaccination and 80 (34.3%) unvaccinated controls. The groups were similar in terms of age, BMI, gravida, parity, smoking status, and contraceptive method (all $p>0.05$), indicating good baseline comparability between cohorts (Table 1).

At baseline, all patients in both groups tested positive for HPV. At the 12-month follow-up, the HPV clearance rate was 70.6% (108/153) in the vaccinated group compared with 52.5% (42/80) in the unvaccinated control group ($p=0.009$). Although reductions in HPV16 and HPV18 positivity, as well as in other high-risk HPV types and multiple HPV infections, were observed in both groups, no differences were found between the groups in these genotype-specific comparisons (all $p>0.05$). The detailed distribution of HPV genotypes and HPV clearance is presented in Table 2.

Evaluation of cervical cytology demonstrated a significantly higher rate of cytological normalization in the vaccinated group at the 12-month follow-up. The proportion of women

Table 1. Baseline demographic and clinical characteristics of the study cohorts

Variable	Vaccinated group (n=153)	Unvaccinated control group (n=80)	*p* value
Continuous variables, mean ± SD			
Age (years)	43.62±9.08	44.06±8.60	0.720
BMI (kg/m²)	29.27±2.87	29.34±2.74	0.859
Gravidy	3.42±2.21	3.48±2.32	0.855
Parity	3.23±2.09	3.08±1.73	0.572
Categorical variables, n (%)			
Smoking (yes)	56 (36.6%)	27 (33.8%)	0.773
Contraceptive method, n (%)			
None	52 (34.0%)	37 (46.2%)	0.088
Condom	32 (20.9%)	18 (22.5%)	0.867
Intrauterine device	52 (34.0%)	19 (23.8%)	0.134
Oral contraceptive pills	17 (11.1%)	6 (7.5%)	0.490

Continuous variables were compared using the independent samples t-test, and categorical variables were compared using Fisher's exact test. A p value <0.05 was considered statistically significant
SD: Standard deviation, BMI: Body mass index

Table 2. Comparison of HPV status at baseline and 12 months within and between the study groups

HPV status	Vaccinated group (n=153)		Unvaccinated control group (n=80)		*p* value*
	Baseline, n (%)	12 months, n (%)	Baseline, n (%)	12 months, n (%)	
HPV negative	0 (0.0%)	108 (70.6%)	0 (0.0%)	42 (52.5%)	**0.009**
HPV16	47 (30.7%)	26 (17.0%)	28 (35.0%)	20 (25.0%)	0.199
HPV18	24 (15.7%)	12 (7.8%)	13 (16.2%)	10 (12.5%)	0.358
Other HPV types	68 (44.4%)	5 (3.3%)	37 (46.2%)	8 (10.0%)	0.068
Co-infection (≥2 HPV types)	14 (9.2%)	2 (1.3%)	2 (2.5%)	0 (0.0%)	0.780

*p value: chi-square test comparing HPV status between the vaccinated and unvaccinated groups at the 12-month follow-up. At baseline, all participants in both groups were HPV-positive
HPV: Human papilloma virus

with normal cytology increased from 17.6% at baseline to 72.5% at 12 months in the vaccinated group, compared with an increase from 12.5% to 26.2% in the unvaccinated control group ($p<0.001$). Likewise, the frequencies of atypical squamous cells of undetermined significance (ASC-US) and LSIL decreased significantly in the vaccinated group compared with the control group (both $p<0.001$). In contrast, no significant difference was observed between the groups in the rate of HSIL, which was uncommon at baseline ($p=0.517$). A detailed comparison of cervical cytology results is presented in Table 3.

At the 12-month follow-up, the vaccinated group demonstrated significantly better outcomes than the unvaccinated control group across all primary outcome measures. All primary outcomes are summarized in Table 4. Complete regression, defined as the simultaneous presence of a negative HPV test and normal cervical cytology, was observed in 53.6% of the vaccinated group compared with only 16.2% of the control group at the 12 month follow-up ($p<0.001$). In contrast, the rate of persistent disease, defined as persistent HPV positivity together with abnormal cervical cytology, was significantly lower in the vaccinated group than in the control group (10.5% vs. 37.5%, $p<0.001$).

Univariable logistic regression analysis was performed in the vaccinated cohort (n=153) to identify baseline predictors of persistent disease, which occurred in only 16 patients (10.5%). The detailed results of the logistic regression analysis are presented in Table 5. Age, BMI, gravida, parity, and smoking status were not significantly associated with the development of persistent disease. In contrast, baseline HPV18 positivity was identified as a significant predictor of persistent disease (OR: 3.97; 95% CI: 1.29-12.24; $p=0.017$). Given the relatively low number of EPV (EPV=2.7), these findings should be interpreted as exploratory and require confirmation in larger studies.

Table 3. Within-group and between-group comparison of cervical cytology results at baseline and 12-month follow-up

Cytology	Vaccinated group (n=153)		Unvaccinated control group (n=80)		*p* value*
	Baseline, n (%)	12 months, n (%)	Baseline, n (%)	12 months, n (%)	
Negative	27 (17.6%)	111 (72.5%)	10 (12.5%)	21 (26.2%)	**<0.001**
ASC-US	49 (32.0%)	19 (12.4%)	30 (37.5%)	28 (35.0%)	**<0.001**
LSIL	67 (43.8%)	20 (13.1%)	39 (48.8%)	31 (38.8%)	**<0.001**
HSIL	9 (5.9%)	3 (2.0%)	1 (1.2%)	0 (0.0%)	0.517

*p value: Chi-square test comparing cervical cytology results between the vaccinated and unvaccinated groups at the 12-month follow-up
ASC-US: Atypical squamous cells of undetermined significance, LSIL: Low-grade squamous intraepithelial lesion, HSIL: High-grade squamous intraepithelial lesion

Table 4. Between-group comparison of clinical outcomes at the 12-month follow-up

Outcome	Vaccinated group (n=153), n (%)	Unvaccinated control group (n=80), n (%)	*p* value
HPV clearance (HPV-negative at 12 months)	108/153 (70.6%)	42/80 (52.5%)	**0.009**
Cytological regression (normal pap smear at 12 months)	111/153 (72.5%)	21/80 (26.2%)	**<0.001**
Complete regression† (HPV-negative and normal cytology)	82/153 (53.6%)	13/80 (16.2%)	**<0.001**
Persistent disease‡ (HPV-positive and abnormal cytology)	16/153 (10.5%)	30/80 (37.5%)	**<0.001**

†Complete regression: defined as both HPV negativity and normal cervical cytology at the 12-month follow-up. ‡Persistent disease (strict definition): Defined as concurrent persistence of HPV positivity and abnormal cervical cytology at the 12-month follow-up. *P* values were calculated using the chi-square test
HPV: Human papilloma virus

Table 5. Univariate logistic regression analysis of factors associated with persistent disease in the vaccinated group (n=153)

Variable	Comparison	OR	95% CI	*p* value
Age	Per 1-year increase	0.98	0.92-1.04	0.504
BMI	Per 1 kg/m^2 increase	1.00	0.83-1.20	0.979
Gravidity	Per 1-unit increase	0.98	0.77-1.24	0.839
Parity	Per 1-unit increase	0.94	0.72-1.22	0.642
Smoking	Yes vs. no	0.77	0.25-2.33	0.639
Baseline HPV18	HPV18 vs. other HPV types	3.97	1.29-12.24	**0.017**

Outcome: Persistent disease (concurrent HPV positivity and abnormal cervical cytology at the 12-month follow-up; n=16, 10.5%). All predictor variables represent baseline values. The 12-month HPV18 variable used in the previous analysis was excluded from the model because of circularity
Events per variable: 16 events/6 variables =2.7. Because the EPV was below the recommended threshold, the findings should be considered exploratory and interpreted with caution
BMI: Body mass index, HPV: Human papilloma virus, OR: Odds ratio, CI: Confidence interval

DISCUSSION

In this retrospective comparative cohort study, we evaluated one-year HPV DNA and cervical cytology outcomes in women with histopathologically confirmed LSIL/CIN1 who received adjuvant nonavalent HPV vaccination compared with an unvaccinated control cohort. Women who received adjuvant HPV vaccination were significantly more likely to have achieved HPV clearance ($p=0.009$), higher rates of normal cervical cytology ($p<0.001$), higher rates of complete regression ($p<0.001$), and lower rates of persistent disease ($p<0.001$) than unvaccinated women. Overall, these findings suggest that adjuvant nonavalent HPV vaccination was associated with significantly better one-year virological and cytological outcomes in women with LSIL/CIN1. However, because of the retrospective observational design, a causal relationship cannot be established.

The natural history of LSIL/CIN1 is generally favorable, with most lesions (57-80%) regressing spontaneously without treatment. Persistent high-risk HPV infection remains the principal determinant of disease progression and represents the main target of HPV vaccination.[1,2] Ostör[13] reported spontaneous regression in approximately 57% of CIN1 lesions, whereas 32% persisted, 11% progressed to CIN3, and only 1% progressed to invasive cervical cancer. Similarly, Gardella et al.[5] reported spontaneous regression rates of 60-80% for LSIL/CIN1 and demonstrated that HPV vaccination was associated with faster HPV clearance and a lower risk of disease progression. In the present study, HPV clearance occurred in 52.5% of unvaccinated women after one year, reflecting the expected natural course of LSIL/CIN1. In contrast, vaccinated women achieved a significantly higher rate of HPV clearance (70.6%, $p=0.009$), supporting the potential role of adjuvant HPV vaccination in promoting viral elimination.

Our findings are consistent with previous studies evaluating adjuvant HPV vaccination in HPV-positive women and those with cervical intraepithelial lesions. In a systematic review including 19,414 women, Pruski et al.[14] reported complete HPV remission in up to 72.4% of vaccinated women compared with 45.7% of unvaccinated controls. Likewise, Palumbo et al.[3] found significantly lower persistent HPV positivity among vaccinated women with CIN1 than among unvaccinated controls (18% vs. 38%, $p=0.0169$). Gardella et al.[5] also demonstrated that vaccination shortened the time to HPV clearance (OR: 1.59, 95% CI: 1.22-2.07), while De Vincenzo et al.[15] reported significantly faster HPV clearance after completion of the three-dose nonavalent vaccination schedule (hazard ratio: 7.80, 95% CI: 4.83-12.60; $p<0.0001$).[5] Together, these findings support the association between adjuvant HPV vaccination and improved viral clearance.

In addition to enhanced HPV clearance, vaccinated women in our study demonstrated significantly better cytological outcomes than unvaccinated controls. At the 12-month follow-up, normal cervical cytology was observed in 72.5% of vaccinated women compared with 26.2% of controls ($p<0.001$). Similarly, ASC-US and LSIL were significantly less frequent in vaccinated women (12.4% vs. 35.0% and 13.1% vs. 38.8%, respectively; both $p<0.001$), whereas no significant difference was observed in HSIL frequency ($p=0.517$). Vaccinated women also had higher complete regression rates (53.6% vs. 16.2%, $p<0.001$) and lower rates of persistent disease (10.5% vs. 37.5%, $p<0.001$). Collectively, these findings suggest that the potential benefit of adjuvant HPV vaccination extends beyond viral clearance and may also be reflected by improved cytological recovery. Similar observations have been reported by Gardella et al.,[5] who demonstrated lower rates of CIN persistence and progression among vaccinated women, and by Pruski et al.,[14] whose systematic review concluded that adjuvant HPV vaccination improved clinical outcomes by enhancing HPV clearance and reducing HPV-related disease progression.[5] Although most previous studies have primarily focused on HPV clearance, our findings indicate that favorable cytological outcomes may also be associated with adjuvant vaccination in women with histologically confirmed LSIL/CIN1.

Baseline HPV18 positivity was the only significant predictor of persistent disease in our cohort (OR: 3.97, 95% CI: 1.29-12.24; $p=0.017$). Persistent HPV18 infection has long been recognized as an important risk factor for cervical carcinogenesis because of its strong association with cervical adenocarcinoma.[16-19] However, the wide CI and the limited number of outcome events indicate that this finding should be interpreted cautiously. Moreover, the low EPV ratio limits the stability of the regression model, and the predictive value of baseline HPV18 positivity should therefore be considered exploratory until confirmed in larger prospective studies.

Study Limitations

This study has several strengths, including the inclusion of only histopathologically confirmed LSIL/CIN1 cases, the use of a contemporaneous unvaccinated control group, and a clinically meaningful definition of persistent disease based on concurrent HPV positivity and abnormal cervical cytology. Nevertheless, several limitations should be acknowledged. The retrospective single-center design precludes causal inference, the 12-month follow-up does not permit assessment of long-term outcomes, and histological confirmation was not available at follow-up. In addition, important potential confounders, including sexual behavior, new sexual partners, condom use, immunological status, and concomitant sexually transmitted infections, could not be evaluated because these variables were not consistently documented in the medical records. These factors should be considered when interpreting the observed associations between adjuvant HPV vaccination and clinical outcomes.

CONCLUSION

Adjuvant nonavalent HPV vaccination was associated with higher HPV clearance, improved cervical cytological outcomes, higher complete regression, and lower persistent disease rates than in unvaccinated women with histopathologically confirmed LSIL/CIN1. These findings support the potential role of adjuvant HPV vaccination as an adjunct to conservative management. Prospective multicenter studies with longer follow-up are needed to confirm these findings.

Ethics

Ethics Committee Approval: Ethical approval for the study was obtained from the Clinical Research Ethics Committee of University of Health Sciences Turkey, Diyarbakır Gazi Yaşargil Training and Research Hospital (approval no: 737, date: 21.11.2025).

Informed Consent: This study was conducted as a retrospective observational case series.

Footnotes

Authorship Contributions

Surgical and Medical Practices: M.A.H., E.Ş., Concept: M.A.H., E.Ş., Design: M.A.H., E.Ş., S.Y., S.B.K., Data Collection or Processing: M.A.H., E.Ş., Analysis or Interpretation: M.A.H., S.C.O., E.Ş., S.Y., M.F.A., S.B.K., Literature Search: M.A.H., E.Ş., Writing: M.A.H., E.Ş., S.Y., M.F.A.

Conflict of Interest: One author of this article, Süleyman Cemil Oğlak, is a member of the Editorial Board of the Anatolian Journal of Obstetrics and Gynecology Research. However, he did not involved in any stage of the editorial decision of the manuscript.

Financial Disclosure: The authors received no financial support for the research, authorship, and/or publication of this article

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