Am J Transl Res 2025;17(4):2992-3000
www.ajtr.org /ISSN:1943-8141/AJTR0162672

Original Article

Risk factors for persistent infection of high-risk HPV in patients with cervical intraepithelial neoplasia

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Received December 14, 2024; Accepted February 16, 2025; Epub April 15, 2025; Published April 30, 2025

Abstract: Objective: To identify the risk factors for persistent HR-HPV infection in patients with cervical intraepithelial neoplasia (CIN). Methods: A total of 312 patients with cervical intraepithelial neoplasia were followed up for six months. Among them, 164 patients with persistent HPV infection during re-examination were categorized into the persistent infection group, while 148 patients with negative HPV results were classified into the negative conversion group. Results: Multivariate logistic regression analyses identified the following independent risk factors for persistent HR-HPV infection: age ≥50 years (95% CI: 3.037-11.447; P<0.001), multiple HPV infections (95% CI: 4.250-18.417; P<0.001), HPV viral load ≥100 (95% CI: 1.529-5.673; P=0.001), reproductive tract inflammation (95% CI: 1.186-4.696; P=0.014), and thyroid dysfunction (95% CI: 8.346-17.207; P<0.001). A prediction model was developed based on the logistic regression analysis: Logit(P) = -102.56 + (age × 1.774) + (HPV multiple infections × 2.180) + (HPV viral load ≥100 × 1.080) + (reproductive tract inflammation × 0.859) + (thyroid dysfunction × 3.650). Receiver operating characteristic (ROC) curve analysis showed an area under the curve (AUC) of 0.800 for the model in predicting persistent high-risk HPV infection, with sensitivity of 81.00% and specificity of 79.46%. Conclusion: Age ≥50 years, multiple HPV infections, HPV viral load ≥100, reproductive tract inflammation, and thyroid dysfunction are independent risk factors for persistent high-risk HPV infection in patients with CIN.

Keywords: Cervical intraepithelial neoplasia, high-risk type, human papillomavirus, persistent infection, risk factor

Introduction

Cervical cancer is a prevalent malignancy in the female reproductive system, ranking fourth among all female cancers, only after breast cancer, colorectal cancer and lung cancer [1]. In 2020, approximately 600,000 new cases of cervical cancer were reported worldwide, accounting for 6.5% of all cancer cases, with about 340,000 deaths, representing 7.7% of total cancer-related deaths [2]. The incidence and mortality rates of cervical cancer are still increasing, with a rising proportion of cases among younger women, posing a significant threat to women's health [3]. Cervical intraepithelial neoplasia (CIN) is a precancerous lesion closely associated with cervical cancer [4]. Women with a history of high-grade CIN are five times more likely to develop cervical cancer compared to those without such a history. Studies indicate that 20%-30% of patients with CIN grade II may progress to cervical cancer within 10 to 20 years [5-7]. Early detection, diagnosis, and timely treatment of CIN are crucial for the prevention and management of cervical cancer.

Human papillomavirus (HPV) infection is the primary cause of both cervical cancer and CIN. Persistent infection with high-risk HPV (HR-HPV) is a key factor in the progression of CIN to cervical cancer [8]. Most HPV infections are asymptomatic or subclinical, but persistent infection can lead to severe outcomes. While the immune system clears the virus in 8 to 10 months in most cases, approximately 10%-15% of patients experience persistent HPV infection, which can result in precancerous cervical lesions and, eventually, invasive cancer [9, 10]. Factors such as a weakened immune system [11], multiple sexual partners [12], early sexual activity [13], smoking [14], and genetic predisposition [15]

https://doi.org/10.62347/GGVR2248

contribute to the persistence of HR-HPV infection in CIN patients.

Persistent infection with high-risk HPV is a crucial factor in the development and progression of CIN. Previous studies have explored various risk factors, including viral genotypes, host immune responses, environmental influences, and behavioral factors [11-15]. However, these studies primarily focused on single-factor analyses, lacking comprehensive evaluations of multifactorial interactions. Additionally, many studies are limited by short follow-up durations or specific populations, making it difficult to fully elucidate the complex mechanisms driving persistent HPV infection. Furthermore, research on the risk of persistent HPV infection following therapeutic interventions remains relatively scarce, particularly in terms of integrating multidimensional factors such as immune status, treatment modalities, and social behaviors.

Utilizing a large-scale patient dataset and long-term follow-up, this study is the first to systematically evaluate the interactions of multiple risk factors for persistent high-risk HPV infection, with a particular focus on the dynamic changes of various factors after therapeutic interventions. Compared with previous studies, this research not only introduces a multivariate analysis model to significantly enhance the reliability of the results but also includes a broader and more diverse population, addressing gaps in the existing literature. The novelty of this study lies in its comprehensive analysis of viral characteristics, host factors, and external interventions, proposing a clinically relevant risk assessment model that offers new insights and practical support for precise management and personalized treatment strategies for CIN patients.

Methods and materials

Study design and population

The retrospective study was conducted from January 2020 and August 2024 at Tongzhou Maternal & Child Health Hospital in Beijing. This study was reviewed and approved by the hospital's medical ethics committee. A total of 312 patients with CIN underwent a six-month follow-up reexamination. Of these, 164 patients who still tested positive for HPV during follow-up were assigned to the persistent infection group, while 148 patients whose HPV results turned negative were placed in the negative conversion group.

Inclusion criteria: 1) Diagnosis confirmed by pathological examination, in accordance with the "Standardized Diagnosis and Treatment Guidelines for Cervical Cancer and Pre-cancerous Lesions (Trial)" [16]; 2) Age >18 years; 3) Positive HPV DNA test; 4) Complete clinical information available.

Exclusion criteria: 1) History of other malignancies or severe organ dysfunction; 2) Immunodeficiency diseases; 3) Mental disorders due to psychiatric conditions; 4) Pregnant or lactating women; 5) Sexually transmitted diseases, including syphilis and AIDS; 6) Liver, heart, or kidney dysfunction; 7) History of hysterectomy; 8) Recent (within 3 months) use of immunosuppressants or hormones; 9) Recent (within 2 weeks) use of antibacterial drugs.

Definition of persistent HPV infection

Persistent HPV infection is defined as the presence of the same HPV type in two or more HPV tests conducted at intervals of 6 to 12 months. However, no official guideline currently defines persistent HPV infection following CIN treatment. Hoffman SR et al. [17] proposed that persistent HPV infection after CIN treatment refers to HPV infection detected both before and during treatment, which continues to be present after treatment ends. This type of infection is classified into three categories: (1) Comprehensive persistent HPV infection: HPV infection of any type combination detected at two consecutive measurement points; (2) Persistent HR-HPV infection: High-risk HPV (HR-HPV) infection detected at two consecutive measurement points; (3) Specific typed persistent HPV infection: The same HPV type is detected at two consecutive measurement points, which can include the preoperative HPV detection and the first follow-up HPV test after treatment. Based on these definitions, in this study, persistent HPV infection is defined as the presence of the same HPV type in a patient at two or more time points within 12 months following LEEP surgery.

Data collection

HPV detection: Patients were positioned in the lithotomy position, and a dry cotton ball was

used to wipe away cervical secretions. A cervical brush was inserted deeply into the internal cervix os, ensuring it closely adheres to the mucosa of the transitional zone. The brush is rotated counterclockwise for four complete rotations. After removal, the brush head was detached and placed in a preservation solution. DNA was extracted using a cell lysis solution, and the second-generation hybrid capture method was employed for HPV detection. A microplate reader was used to measure the light generated by each sample. The results were evaluated based on the ratio of relative light unit (RLU) to the control threshold (CO). An RLU/CO ratio ≥1 indicates a positive HPV infection. The test kit, purchased from Digene Company (USA), detected 13 high-risk HPV genotypes (HPV16/18/31/33/35/39/45/51/52/56/58/59/68). Multiple HPV infections are indicated by the presence of ≥2 HPV genotypes. Following LEEP surgery, the pathological tissue from the surgical margin was examined for HPV infection using the same hybrid capture method.

Vaginal flora detection: During follow-up visits, patients are placed in the lithotomy position, and a speculum is inserted to expose the cervix. Two sterile cotton swabs are used to collect secretions from the 1/3 section of the vaginal sidewall, rotating 3 to 5 times. No intravaginal treatments or irrigation are allowed 72 hours prior to collection. The first cotton swab is used to measure vaginal pH with a precision pH test strip (range 3.8-5.4). The second cotton swab is used to smear secretions onto a glass slide, which is then dried, fixed, and stained with Gram stain. The vaginal flora is examined under an oil immersion microscope (10 × 100 magnification). The density, diversity, and dominant bacteria of the vaginal flora are evaluated according to the "Expert Consensus on the Clinical Application of Vaginal Microecological Evaluation" [18].

(1) Density of vaginal flora: Grade I: 1-9 bacteria per field; Grade II: 10-99 bacteria per field; Grade III: ≥100 bacteria per field; Grade IV: Bacteria aggregate or densely cover epithelial cells. Grades II to III indicate normal vaginal flora density.

(2) Diversity of vaginal flora: Grade I: 1 to 3 bacterial types; Grade II: 4-6 bacterial types; Grade III: 7 to 9 bacterial types; Grade IV: ≥10 bacterial types. Grades II to III indicate normal diversity.

(3) Dominant bacteria: Normal vaginal microbial flora is indicated with Lactobacillus as the dominant bacteria observed.

Detection of blood biochemical indicators: Blood samples are collected using EDTA-K2 anticoagulant vacuum tubes. For pregnant females, 5 ml of blood is drawn from the cubital vein during antenatal check-ups. The collected blood is divided, labeled, and stored at 4°C before being centrifuged at 4000 rpm for 10 minutes. The serum is then stored at -70°C. Blood biochemical indicators are measured using the ACL-200 fully automatic coagulation analyzer (Coulter, USA), with reagents including Hemosil™ Reference Emulsion, Hemosil™ APTT Lyophilized Silica, Test™ PT-Fibrinogen HS, Hemosil Calibration Plasma, and Hemosil™ Calcium Chloride.

Outcome measurements

The primary outcomes included laboratory data collected upon admission, such as peripheral blood cellular and humoral immunity, renal function, thyroid function, and blood lipid levels. The secondary outcomes included general patient information collected through the hospital's case query system, including age, diabetes mellitus, smoking history, and hypertension.

Statistical methods

Data analysis was performed using SPSS 20.0 statistical software. The measurement data were expressed as the mean ± standard deviation, and independent t-tests were used for comparison between groups. Categorical data were expressed as percentages (%), and chi-square tests were used for group comparisons. Multivariate Logistic regression analyses were conducted to identify factors influencing persistent high-risk HPV infection in CIN patients. A predictive model for persistent HR-HPV infection was further developed based on the screened risk factors, and its predictive performance was assessed using Receiver Operating Characteristic (ROC) curve analysis. A *P*-value <0.05 was considered statistically significant.

Table 1. Comparison of general information between the two groups

	HPV persistent infection group (n=164)	HPV conversion group (n=148)	t/χ^2	*P*
Age (years)	29.90±3.83	30.54±3.52	1.541	0.124
BMI	20.70±1.52	20.82±1.05	0.752	0.453
Smoking history	84 (51.2%)	86 (58.1%)	1.489	0.222
History of alcohol consumption	81 (49.4%)	62 (41.9%)	1.762	0.184
Marital status			0.028	0.867
Married	132 (80.5%)	118 (79.7%)		
Unmarried or divorced	32 (19.5%)	30 (20.2%)		
Age at first sexual behavior	20.65±2.78	21.53±2.57		
Coronary heart disease	12 (7.3%)	12 (8.1%)	0.069	0.793
Diabetes	14 (8.5%)	10 (6.8%)	0.347	0.556
Hypertension	12 (7.3%)	10 (6.8%)	0.037	0.847
Anemia	8 (4.9%)	6 (4.1%)	0.123	0.726
Hypoproteinemia	8 (4.9%)	7 (4.7%)	0.004	0.951
Reproductive tract inflammation	85 (51.8%)	30 (20.2%)	33.292	<0.001
Number of pregnancies	1.70±0.39	2.48±0.94	9.690	<0.001
HPV viral load (RLU/CO)	156.08±22.16	113.35±13.34	20.366	<0.001
Multiple HPV infection	132 (80.5%)	55 (37.2%)	60.813	<0.001
Number of vaginal deliveries	3.06±0.58	1.71±1.04	14.299	<0.001

Note: HPV: Human Papilloma virus; RLU/CO: Relative Light Unit/Control threshold; BMI: body mass index.

Results

Comparison of general characteristics between the two groups

No significant differences were observed between the two groups in general clinical data such as age, BMI, smoking, alcohol consumption, marital status, age at first sexual intercourse, coronary heart disease, diabetes, hypertension, anemia, and hypoproteinemia (all P>0.05). However, significant differences were found in reproductive tract inflammation, number of pregnancies, HPV viral load (RLU/CO), multiple HPV infections, and number of vaginal deliveries (all P<0.05) (**Table 1**).

Comparison of peripheral blood cellular immunity and humoral immunity between the two groups

The CD3 levels were (61.98±3.38) in the persistent HPV infection group and (69.92±3.76) in the HPV conversion group (P<0.001). The CD4 levels were (18.41±1.44) in the persistent HPV infection group and (23.87±2.04) in the HPV conversion group (P<0.001). The CD8 levels were (28.04±5.50) in the persistent HPV infection group and (30.15±7.11) in the HPV conversion group (P<0.001). The CD4/CD8 ratio in the persistent HPV infection group was (0.57±0.09), while that in the HPV conversion group was (0.95±0.14) (P<0.001). Furthermore, the Immunoglobulin A (IgA), IgG, and IgM levels were (0.56±0.10), (5.00±0.67) and (0.51±0.06) in the persistent HPV infection group, significantly lower than (0.61±0.11), (6.93±0.54) and (0.54±0.09) in the HPV conversion group (all P<0.001) (**Table 2**).

Comparison of renal function between the two groups

There were no significant differences between the two groups in renal function parameters, including urea, creatinine, urea/creatinine ratio, and uric acid levels (all P>0.05) (**Table 3**).

Comparison of thyroid function between the two groups

Significant differences were observed between the groups in levels of free triiodothyronine (FT3), free thyroxine (FT4), thyroid stimulating hormone (TSH), and thyroglobulin (all P<0.05). However, no significant differences were noted in triiodothyronine (T3), thyroxine (T4), and thy-

Table 2. Comparison of peripheral blood cellular and humoral immunity parameters between the two groups

Indexes	HPV Persistent Infection Group (n=164)	HPV Conversion Group (n=148)	t	P
CD3	61.98±3.38	69.92±3.76	19.671	<0.001
CD4	18.41±1.44	28.37±2.04	50.165	<0.001
CD8	28.04±5.50	30.15±1.71	4.468	<0.001
CD4/CD8	0.57±0.09	0.95±0.14	28.890	<0.001
IgA	0.56±0.10	0.61±0.11	4.727	<0.001
IgG	5.00±0.67	6.93±0.54	27.893	<0.001
IgM	0.51±0.06	0.54±0.09	3.495	0.001

Note: HPV: Human Papilloma virus; CD3: Cluster of Differentiation 3; CD4: Cluster of Differentiation 4; CD8: Cluster of Differentiation 8; CD4/CD8: The ratio of CD4 to CD8 T cells; IgA: Immunoglobulin A; IgG: Immunoglobulin G; IgM: Immunoglobulin M.

Table 3. Comparison of renal function indices between the two groups

Index	HPV Persistent Infection Group (n=164)	HPV Conversion Group (n=148)	T	p
Urea (mmol/L)	3.78±2.12	3.91±1.71	0.583	0.561
Creatinine (μmol/L)	53.24±4.37	53.37±4.65	0.258	0.796
Urea/Creatinine	0.12±0.02	0.12±0.02	0.724	0.470
Uric Acid (μmol/L)	256.62±44.72	258.14±34.54	0.334	0.738

Note: HPV: Human Papilloma virus.

roid globulin antibody levels between the two groups (all P>0.05) (**Table 4**).

Comparison of blood lipid levels between the two groups

There were no significant differences between the two groups in blood lipid levels, including total cholesterol (CHO), triglycerides (TG), high-density lipoprotein (HDL), and low-density lipoprotein (LDL) (all P>0.05) (**Table 5**).

Multivariate analysis

The results of the multivariate logistic regression analysis showed that age ≥50 years (95% CI: 3.037-11.447; P<0.001), multiple HPV infections (95% CI: 4.250-18.417; P<0.001), HPV viral load ≥100 (RLU/CO) (95% CI: 1.529-5.673; P=0.001), reproductive tract inflammation (95% CI: 1.186-4.696; P=0.014), and thyroid dysfunction (95% CI 8.346-17.207; P<0.001) were independent risk factors for persistent high-risk HPV infection (**Table 6**).

ROC curve analysis

Incorporating the above factors into Logistic regression analysis, the prediction model was developed: Logit(P) = -102.56 + age * 1.774 + HPV multiple infections * 2.180 + HPV viral load ≥100 * 1.080 + reproductive tract inflammation * 0.859 + thyroid dysfunction * 3.650. The ROC curve was used to evaluate the model for predicting persistent high-risk HPV infection. When Logit(P) >12.24, the model showed an AUC value of 0.800, with sensitivity of 81.00% and specificity of 79.46% (**Figure 1**), indicating that the logistic regression model is effective.

Discussion

In our study, we identified age ≥50 years, HPV multiple infections, HPV viral load ≥100, reproductive tract inflammation, and thyroid dysfunction as independent risk factors for persistent high-risk HPV infection in patients with cervical intraepithelial neoplasia (CIN). In addition, the ROC curve analysis confirmed the effective construction of the Logistic regression analysis-based diagnostic model.

We found that older age (≥50 years) is a high-risk factor for persistent high-risk HPV infection in patients with CIN, which is consistent with existing study [19]. As age increases, immune function gradually declines, weakening the body's ability to clear HPV infections. This diminished immune response may reduce the efficiency of recognizing and eliminating the virus, allowing it to persist and establish chronic infection [20, 21]. Additionally, with advancing age, changes in the cervical microenvironment - such as alterations in local cell composition and cytokine levels - may affect the interaction between HPV and host cells, thereby promoting viral persistence [22]. Moreover, cumulative exposure to various risk factors over time, such as multiple sexual partners or a history of sexually transmitted infections,

Table 4. Comparison of thyroid function parameters between the two groups

Index	HPV Persistent Infection Group (n=164)	HPV Conversion Group (n=148)	t	P
T3 (nmol/L)	1.82±0.15	1.79±0.11	1.822	0.069
T4 (nmol/L)	109.76±13.10	110.76±9.42	0.771	0.441
FT3 (pmol/L)	10.53±0.87	6.08±0.38	57.495	<0.001
FT4 (pmol/L)	21.88±4.51	20.11±1.75	4.455	<0.001
TSH (mIU/L)	13.09±4.75	7.13±4.09	11.821	<0.001
Thyroglobulin (ng/mL)	15.76±3.78	12.22±5.64	6.561	<0.001
Thyroid Globulin Antibody (IU/mL)	48.41±4.17	49.01±4.14	1.265	0.207

Note: T3: triiodothyronine; T4: thyroxine; FT3: Free Triiodothyronine; FT4: Free Thyroxine; FSH: Thyroid Stimulating Hormone; HPV: Human Papilloma virus.

Table 5. Comparison of blood lipid levels between the two groups

Index	HPV Persistent Infection Group (n=164)	HPV Conversion Group (n=148)	t	P
CHO (mmol/L)	3.49±1.18	3.61±0.24	1.220	0.223
TG (mmol/L)	1.27±0.57	1.37±0.21	1.939	0.053
HDL (mmol/L)	1.81±0.20	1.77±0.63	1.868	0.063
LDL (mmol/L)	1.77±0.63	1.81±0.28	0.729	0.467

Note: CHO: cholesterol; TG: triglyceride; HDL: high-density lipoprotein; LDL: low-density lipoprotein; HPV: Human Papilloma virus.

may further increase the likelihood of persistent high-risk HPV infection [23]. Age-related hormonal changes could also contribute to the susceptibility and persistence of HPV infection in the cervical epithelium.

Multiple HPV infections were identified as a high-risk factor for persistent high-risk HPV infection in patients with CIN. Different HPV types exhibit varying abilities to adhere to and infect cervical epithelial cells. When multiple HPV types are present simultaneously, they may interact or compete with one another, but some types may have a stronger capacity to establish persistent infection [24]. Furthermore, the body's immune response may struggle to manage multiple infections simultaneously, allowing the viruses to persist over a longer period. Certain HPV types may be more adept at evading immune surveillance or have a higher potential to induce persistent cellular changes. The complex interactions between various HPV types and the host immune system increase the likelihood that multiple infections will lead to a sustained high-risk HPV infection in CIN patients [25-27]. Moreover, genetic and environmental factors may also contribute to the persistence of multiple HPV infections and their impact on lesion progression.

Our study also found that HPV viral load (RLU/CO) ≥100 is a high-risk factor for persistent high-risk HPV infection in patients with CIN. HPV is a key factor in the development of CIN [28]. When the viral load reaches or exceeds 100, it indicates a relatively high HPV presence. This high viral load has several important implications. First, a large quantity of HPV can continuously stimulate cervical epithelial cells, leading to persistent damage and abnormal proliferation, which contributes significantly to the persistence of high-risk HPV infection [29, 30]. Second, a high viral load may impair the normal immune response, making it difficult for the immune system to clear the virus and further promoting persistent infection [31, 32]. Moreover, the elevated HPV concentration could destabilize cellular genes, enhancing the progression of cervical epithelial abnormalities [33].

We also found that reproductive tract inflammation is another risk factor for persistent high-risk HPV infection in patients with CIN. Inflammation in the reproductive tract can disrupt the normal physiological environment of the cervix, impairing local immune function [34]. This weakened immune state makes it difficult for the body to effectively clear the high-risk HPV virus, allowing it to persist and replicate. Additionally, the inflammatory response may lead to changes in the cervical mucosa, such as increased permeability and altered epithelial barrier function, which facilitates the adherence, invasion, and establish-

Table 6. Multivariate logistic regression analysis of persistent high-risk HPV infection

Independent Variable	B	SE	Wald-χ^2	P	95% CI
Age ≥50 years	1.774	0.338	27.485	<0.001	5.896 (3.037-11.447)
HPV Multiple Infections	2.180	0.374	33.966	<0.001	8.847 (4.250-18.417)
HPV Viral Load ≥100 RLU/CO	1.080	0.334	10.436	0.001	2.946 (1.529-5.673)
Reproductive Tract Inflammation	0.859	0.351	5.987	0.014	2.360 (1.186-4.696)
Thyroid Dysfunction	3.650	0.781	21.784	<0.001	38.566 (8.346-17.207)

Note: HPV: Human Papilloma virus; RLU/CO: Relative Light Unit/Control threshold.

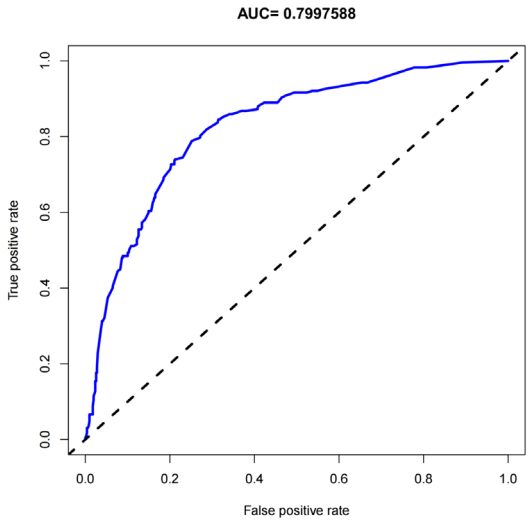

Figure 1. ROC curve of predictive model for persistent high-risk HPV infection in patients with cervical intraepithelial neoplasia. Note: HPV: Human Papilloma virus; ROC: Receiver Operating Characteristic.

ment of persistent HPV infection [35, 36]. Moreover, the inflammatory mediators and cytokines released during inflammation can also influence the interaction between HPV and host cells, further promoting the persistence of high-risk HPV infection.

Interestingly, we found that thyroid dysfunction is another risk factor for persistent high-risk HPV infection in CIN patients. Thyroid hormones play a vital role in regulating the immune system. Abnormal thyroid functions, such as hypothyroidism or hyperthyroidism, can disrupt the normal immune balance and function [37]. A weakened immune system may reduce the ability to clear HPV effectively, allowing the virus to persist and establish a chronic infection. Specifically, thyroid disorders can impair the function of immune cells, including T cells and natural killer cells, which are essential for defending against HPV and preventing its persistence. Moreover, thyroid dysfunction may also alter the cervical microenvironment. Alterations in hormonal levels and metabolic states associated with thyroid abnormalities may create an environment more favorable for the survival and replication of HPV [38]. This, in turn, contributes to the persistence of high-risk HPV in the cervical epithelium of patients with CIN.

This study has certain limitations. First, as a retrospective analysis, it is susceptible to selection bias. For example, the inclusion of only patients who underwent specific treatments may limit the generalizability of the findings to broader populations. Second, some potentially important factors, such as host genetic susceptibility, viral molecular mechanisms, and long-term changes in lifestyle, were not fully incorporated into the analytical model. Furthermore, the dynamic process of HPV infection and its interactions with the host immune response were not thoroughly investigated. Finally, the data for this study were derived from a single region. Although the sample size was relatively large, its representativeness in terms of ethnicity, socioeconomic background, and geographical variation might be insufficient. Future studies should consider multicenter prospective cohort designs to gather more comprehensive and balanced population samples, enhancing the external applicability and reliability of the findings.

In conclusion, age ≥50 years, multiple HPV infections, HPV viral load ≥100, reproductive tract inflammation, and thyroid dysfunction are independent risk factors for persistent high-risk HPV infection in patients with CIN. Clinically, timely interventions should be implemented to reduce the risk of persistent high-risk HPV infection and the potential progression to cervical lesions. Early detection and

treatment of lesions are crucial to improving patient outcomes and ultimately saving women's lives.

Disclosure of conflict of interest

None.

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