Invest Clin 66(2): 131 - 146, 2025 https://doi.org/10.54817/IC.v66n2a01

Correlation between human papillomavirus infection and vaginal microecological environment, and the effect of *Lactobacillus* vaginal capsules combined with recombinant human interferon α-2b gel on human papillomavirus infection.

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Keywords: human papillomavirus infection; vaginal microecology; bacterial vaginosis; *Lactobacillus* vaginal capsule; recombinant human interferon α-2b gel.

Abstract. This study mainly analyzed the correlation between human papillomavirus infection (HPV) and vaginal microecological environment and explored the effect of *Lactobacillus* vaginal capsule combined with recombinant human interferon α-2b gel on HPV infection. Five hundred patients who underwent a gynecological examination in our hospital from June 2021 to June 2023 were selected and divided into HPV-positive and HPV-negative groups. Relative to the HPV-negative group, the HPV-positive group presented a higher abnormal rate of *Lactobacillus*, catalase, cleanliness, neuraminidase and proline aminopeptidase ($p<0.05$) and a higher positive rate of bacterial vaginosis (BV) ($p<0.05$). Multivariate logistic regression analysis showed that catalase, proline aminopeptidase and BV were risk factors for HPV infection ($p<0.05$). In addition, 180 HPV-positive patients were randomly divided into a control group (CG) and an observation group (OG). The CG was given recombinant human interferon α-2b gel, and the OG was treated with recombinant human interferon α-2b gel plus a *Lactobacillus* vaginal capsule. Relative to the CG, the OG presented a higher total effective rate ($p<0.05$), lower inflammation ($p<0.01$), better immune function ($p<0.01$), and a higher proportion of grade II-III vaginal flora density and grade II-III vaginal flora diversity ($p<0.001$). Collectively, HPV is significantly correlated with the vaginal microecological environment, and catalase, proline aminopeptidase and BV were closely related to HPV infection. In addition, *Lactobacillus* vaginal capsule plus recombinant human interferon α-2b gel has practical clinical efficacy, which can reduce inflammation, promote immune function, improve vaginal microecological environment, and is safe in the treatment of patients with HPV infection.

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Correlación entre la infección por el virus del papiloma humano y el entorno microecológico vaginal, y el efecto de cápsulas vaginales de *Lactobacillus* combinada con gel de interferón α-2b humano recombinante sobre la infección por el virus del papiloma humano.

Invest Clin 2025; 66 (2): 131 – 146

Palabras clave: infección por virus del papiloma humano; microecología vaginal; vaginosis bacteriana; cápsula vaginal de *Lactobacillus;* gel recombinante humano de interferón α-2b.

Resumen. Este estudio analizó principalmente la correlación entre la infección por el virus del papiloma humano (VPH) y el entorno microecológico vaginal, y exploró el efecto de cápsulas vaginales de *Lactobacillus* combinadas con gel de interferón humano recombinante α-2b sobre la infección por VPH. Se seleccionaron 500 pacientes que se sometieron a examen ginecológico en nuestro hospital de junio de 2021 a junio de 2023 y se las dividió en un grupo positivo para VPH y un grupo negativo para VPH. En relación con el grupo VPH negativo, el grupo VPH positivo presentó mayor tasa anormal de *Lactobacillus*, catalasa, neuraminidasa, prolina aminopeptidasa y limpieza ($p<0,05$) y mayor tasa positiva de vaginosis bacteriana (BV) ($p<0,05$). El análisis multivariado de regresión logística mostró que la catalasa, la prolina aminopeptidasa y la BV fueron factores de riesgo para la infección por VPH ($p<0,05$). Además, 180 pacientes VPH positivos se dividieron aleatoriamente en un grupo control (GC) y un grupo de observación (OG). Al CG se le administró gel recombinante humano de interferón − -2b, y al OG se le administró gel recombinante humano de interferón − -2b más cápsulas vaginales de *Lactobacillus*. En relación con el GC, el OG presentó mayor tasa efectiva total ($p<0,05$), menor inflamación ($p<0,01$), mejor función inmune ($p<0,01$) y mayor proporción de densidad de flora vaginal de grado II-III y diversidad de flora vaginal de grado II-III ($p< 0,001$). Colectivamente, el VPH se correlaciona significativamente con el entorno microecológico vaginal, y la catalasa, la prolina aminopeptidasa y el BV se relación estrechamente con la infección por VPH. Además, las cápsulas vaginales de *Lactobacillus* más el gel de interferón α-2b recombinante humano tienen eficacia clínica efectiva, lo que puede reducir la inflamación, promover la función inmune, mejorar el entorno microecológico vaginal, y es seguro en el tratamiento de pacientes con infección por VPH.

Received: 29-12-2024 Accepted: 12-04-2025

INTRODUCTION

Cervical cancer is a serious threat to the majority of women's health. In developing countries, its incidence is second only to breast cancer, ranking second in female malignant tumors, and is the most common female reproductive system tumor [1]. According to the cancer report released by the International Agency for Research on Cancer under the World Health Organization, in 2020, about 604,000 women around the

world were diagnosed with cervical cancer, and about 342,000 women died from the disease, among which the number of cervical cancer cases in China was about 110,000, and the number of deaths was 60,000 [2]. Etiological studies have found that persistent infection by human papillomavirus (HPV) is a significant factor in cervical cancer [3]. HPV is a kind of virus which can be classified into low-risk human papillomavirus (LR-HPV) and high-risk human papillomavirus (HR-HPV) according to the strength of the pathogenicity or carcinogenic risk of HPV [4]. HPV is highly host-specific and easily infects human epidermal and mucous squamous epithelium, mainly through sexual transmission[5]. HPV infection is the leading cause of most cervical cancer, anal and oropharyngeal invasive cancers and preinvasive lesions, and is also the cause of genital warts (*condyloma acuminatum*) and recurrent respiratory papillomatosis [6]. Therefore, the prevention of HPV infection and intervention in the disease process after HPV infection are the key to reducing the incidence and mortality of cervical cancer.

There is evidence that maintaining the balance of vaginal microecology plays an important role in preventing female reproductive system infection, and when the vaginal microecology is destroyed, it may lead to cervical lesions [7]. Under normal circumstances, the vaginal microecology is in a state of dynamic balance. When the loss of this dynamic balance, the immune system of the vaginal mucosa, is damaged, and foreign microorganisms are more likely to invade the reproductive tract and cause inflammation [8]. Studies have shown that changes in the vaginal microenvironment, such as vaginal douching, bacterial vaginosis (BV), as well as sexually transmitted infections, are thought to be cofactors in the persistence of HPV infection [9]. Studies have also found that reconstructing vaginal microecological homeostasis can reduce the risk of HPV infection [10].

Antiviral drugs are commonly used in clinical treatment for patients with HPV infection, such as recombinant human interferon α-2b gel, which can inhibit the replication of the virus and increase the immune regulation of lymphocytes to specific cytotoxicity after medication, thus playing a particular therapeutic effect [11]. However, with the single use of antiviral drugs, the drug interacts with the vaginal environment and affects the overall effect. *Lactobacillus* vaginal capsules is a kind of microecological preparation whose main ingredient is living intestinal streptococcus; it is a common drug to treat bacterial disorder vaginosis and can play a role in the decomposition of lactic acid produced by sugar and regulate the pH of the vagina [12].

In our study, we intended to explore the correlation between HPV infection and the vaginal microecological environment and the effect of *Lactobacillus* vaginal capsules combined with recombinant human interferon α-2b gel on HPV infection.

PATIENTS AND METHODS

Patients

Five hundred patients who underwent gynecological examination in the gynecological outpatient department of our hospital from June 2021 to June 2023 were selected as the study participants. Inclusion criteria: (1) Non-pregnant and lactation period; (2) Regular menstrual cycle; (3) No vaginal bleeding; (4) No serious diseases of other systems; (5) No history of vaginal douching treatment and other vaginal operations within three days; (6) No sexual intercourse within three days; (7) No drugs taken within one month, no abnormalities in cervical cytology within one year. Exclusion criteria: (1) Patients had other systemic diseases; (2) Patients had no sexual history. The hospital Medical Ethics Committee approved this study, and all patients signed an informed consent. According to the HPV test results, 180 cases were divided into the HPV-positive group and 320 cases were the HPV-negative group. The HPV-positive group was 22-65

years, with a median age of (37.36±3.75) years. The HPV-negative group was 23-68 years, with a median age of (37.28±3.67) years. The two groups exhibited no significant difference in age ($p>0.05$). In addition, 180 HPV-positive patients were randomly divided into a control group (CG) and an observation group (OG); each group had 90 cases. The control group was 22-68 years old, with a median age of (38.36±3.78) years. The observation group was aged 23-65, with a median age of (37.45±3.69) years. The two groups exhibited no significant difference in age ($p>0.05$).

HPV detection

All 500 enrolled patients underwent HPV testing during the gynecological examination phase of the study, including the 180 patients later identified as HPV-positive. HPV detection was carried out using the second-generation Hybrid Capture 2 (HC2) DNA assay (Qiagen Digene, USA), a gold-standard, FDA-approved diagnostic method for detecting high-risk and low-risk human papillomavirus DNA in cervical samples. This method employs RNA probes complementary to 13 high-risk (e.g., HPV16, 18, 31, 33) and five low-risk HPV genotypes. Sample collection involved using a cervical sampler brush (Digene), which was rotated clockwise three times at the cervical ostium to obtain epithelial cells. The brush was then placed into the HPV DNA collection medium and stored at 4°C. Samples were processed according to the manufacturer's protocol. In the HC2 system, RNA:DNA hybrids formed during hybridization were captured and detected via chemiluminescence, and results were interpreted quantitatively based on a Relative Light Unit (RLU) ratio ≥1.0, with values above this threshold considered HPV-positive. This method was used consistently across all participants to ensure homogeneity of diagnostic criteria, including initial diagnosis in the 180 HPV-positive patients and post-treatment evaluation of viral clearance. All HPV-positive patients (n=180) were subjected to this post-treatment re-evaluation 4–6 weeks after completion of therapy to minimize false negatives due to transient viral suppression. This re-evaluation enabled an accurate assessment of HPV viral persistence or clearance.

HPV Detection Pre- and Post-Treatment
Vaginal microecological examination

The patient was instructed to take the bladder lithotomy position, and the disposable vaginal speculum was slowly inserted into the vagina along the lateral posterior wall of the vagina. As the speculum went deeper, the speculum was turned straight and slowly opened to expose the vaginal wall, fornix, and cervix fully. The vaginal mucus and cells were gently scraped from the upper 1/3 side of the vagina's wall with a disposable sterile cotton swab and then placed in a disposable sterile test tube for sealing. The samples were immediately sent to our hospital's laboratory.

Diagnostic criteria of vaginal microecology

The results were determined by the vaginitis five-test kit (Autobio, Zhengzhou, China). Light yellow or no color of catalase was positive (+), indicating the presence of a small amount of *Lactobacillus*; light red was weakly positive (±), indicating the presence of moderate *Lactobacillus*, and red or purple-red was negative (-); indicating the presence of a large number of *Lactobacillus*. Leucocyte esterase displayed blue for positive (+, ++, +++), light blue for weak positive (±), and no color or light color for negative (-).

Neuraminidase displayed red, purple, blue, brown or black for positive (+), light red for weak positive (±), and no color or orange for negative (-). Proline aminopeptidase showed positive (+) in yellow, weakly positive (±) in light yellow, and negative (-) in no color or light color. Acetylglucosaminidase showed yellow as positive (+), light yellow as weakly positive (±), and no color

or light color as negative (-). Proline aminopeptidase showed positive, and acetylglucosaminidase showed negative, indicating positive BV. Proline aminopeptidase showed positive with a pH ≥4.8, indicating positive trichomonas vaginitis (TV), and proline aminopeptidase showed positive with a PH ≤4.5, indicating positive vulvovaginal candidiasis (VVC). The PH color from yellow-cyan-green-blue indicated a change from 3.8 to 5.4, and the PH value was obtained against the colorimetric card. Vaginal PH of 3.8 to 4.5 was normal. Cleanliness I and II were normal. A large or medium amount of *Lactobacillus* was normal. The negative and weak positive of catalase and leucocyte esterase were normal. Negative neuraminidase, proline aminopeptidase, and acetylglucosaminidase were normal. Negative BV, candida, trichomonas was normal. If there was any abnormality, the vaginal microecology was in an unbalanced state.

Treatment methods

The control group was given recombinant human interferon α-2b gel (Zhaoke Pharmaceutical (Hefei) Co., LTD.; specification: 100,000 IU/g, 5 g/ branch). After the menstrual period was clear, the vulva was cleaned every night, and 1 g of gel was applied to the vaginal dome using a disposable thruster. Seven to ten times was used in a menstrual cycle as a course of treatment, for a total of three courses of treatment.

The observation group was treated with recombinant human interferon α-2b gel combined with *Lactobacillus* vaginal capsules. The treatment method of recombinant human interferon α-2b gel was the same as that of the control group. A *Lactobacillus* vaginal capsule (Xi'an Zhenghao Bio-pharmaceutical Co., LTD., specification: 0.25 g: 6 million live lactic acid bacteria) was added in the recombinant human interferon α-2b gel administration daily, transvaginal medication, two capsules/time, one time/day, with the same treatment course as the control group. During the treatment period, both groups suspended sexual activity and prohibited from vaginal irrigation.

Observation indicators

1. **Clinical efficacy**: a. Obvious effect: After treatment, the characteristics of vaginal secretions returned to normal, all symptoms disappeared, HPV was negative; b. Effective: After treatment, the characteristics and symptoms of the vaginal secretions were significantly improved, and HPV was negative; c. Ineffective: After treatment, HPV was still positive, and symptoms and vaginal secretion traits did not improve or even worsen. Total effective rate = (Obvious effect + Effective) Number of cases/Total cases ×100%.
2. **Inflammation**: 3 mL fasting venous blood samples were taken from the patient and centrifuged for 10 min at a rotational speed of 3000 r/min and a radius of 15 cm. After that, an automatic biochemical analyzer measured the levels of tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6).
3. **Immune function**: Fasting venous blood was collected and centrifuged in parallel according to (2). The levels of $CD4^+$ and $CD8^+$ were measured by flow cytometry, and the ratio of $CD4^+/CD8^+$ was calculated.
4. **Vaginal microecology**: A sterile cotton swab was used to collect secretions on 1/3 of the vaginal wall and detect them under a microscope. The *density of vaginal flora* was divided into grade I (average bacterial count of field ≤9), grade II (average bacterial count of field 10-99), grade III (average bacterial count of field ≥100), and grade IV (bacteria gathered into clusters or densely covered mucosal epithelial cells). Among them, grade II and III were considered as normal concentrations of vaginal flora. *Vaginal flora diversity* was divided into grade I (identify 1 ~ 3 kinds of bacteria), grade II (identify 4 ~ 6 kinds

of bacteria), grade III (identify 7 ~ 9 kinds of bacteria), grade IV (identify ≥10 kinds of bacteria), of which grade II and grade III were considered as normal vaginal flora diversity.

5. **The occurrence of adverse reactions**, including a burning sensation of the vulva, pruritus and gastrointestinal discomfort in the two groups were recorded.

Statistical analysis

The data were analyzed using SPSS 22.0 statistical software. Measurement data were expressed as ($\bar{x}\pm s$), and the t-test was adopted for comparison. Statistical data were expressed as n (%), and the χ^2 test was used for comparison. A logistic regression model was used to analyze the influencing factors. The difference was statistically significant at $p<0.05$.

RESULTS

Comparison of vaginal microecology between two groups

As Table 1 reveals, relative to the HPV-negative group, the HPV-positive group presented a higher abnormal rate of *Lactobacillus*, catalase, cleanliness, neuraminidase and proline aminopeptidase ($p<0.05$). However, no difference was exhibited in the abnormal rates of pH value, leukocyte esterase and acetylglucosaminidase between the two groups ($p>0.05$).

Comparison of positive rates of Bacterial vaginosis, Trichomonas vaginitis and Vulvovaginal candidiasis between the two groups

As Table 2 indicates, relative to the HPV-negative group, the HPV-positive group presented a higher positive rate of BV ($p<0.05$). However, no differences were exhibited in the positive rates of TV and VVC between the two groups ($p>0.05$).

Correlation between HPV infection and vaginal microecology

According to the results of univariate analysis between the two groups, *Lactobacillus*, catalase, cleanliness, neuraminidase, proline aminopeptidase and BV were taken as covariables, and HPV infection as dependent variables, and a multivariate logistic regression analysis was performed. The multivariate logistic regression analysis results showed that catalase, proline aminopeptidase and BV were closely related to HPV infection ($p<0.05$), and were risk factors for HPV infection, as shown in Table 3.

Clinical efficacy between two groups

Relative to the CG, the OG presented a higher total effective rate ($p<0.05$, Table 4).

Inflammation between the two groups

Before therapy, there were no differences in levels of inflammatory markers between the two groups ($p>0.05$). After therapy, TNF-α and IL-6 levels were declined in the two groups ($p<0.01$). Importantly, relative to the CG, the OG presented lower levels of the above inflammatory markers after therapy ($p<0.01$, Fig. 1).

Immune function between two groups

Prior to therapy, there were no differences in levels of immune function indexes between the two groups ($p>0.05$). After therapy, $CD4^+$ and $CD4^+/CD8^+$ levels were elevated, while $CD8^+$ levels declined in the two groups ($p<0.01$). Importantly, relative to the CG, the OG presented better improvements in the above immune function indexes after therapy ($p<0.01$, Fig. 2).

Vaginal microecology between two groups

The proportion of grade II-III vaginal flora density and grade II-III vaginal flora diversity in group 2 were higher than those before treatment ($p<0.001$ and $p=0.002$). Importantly, relative to the CG, the OG presented a higher proportion of grade II-III

Table 1. Comparison of vaginal microecology between two groups.

Index	HPV-negative group (n=320)	HPV-positive group (n=180)	χ^2	p
Lactobacillus			8.638	0.003
Normal	131 (40.94)*	50 (27.78)		
Abnormal	189 (59.06)	130 (72.22)		
Catalase			8.316	0.004
Normal	134 (41.88)	52 (28.89)		
Abnormal	186 (58.12)	128 (71.11)		
pH			0.278	0.598
≤4.5	304 (95.00)	169 (93.89)		
>4.5	16 (5.00)	11 (6.11)		
Cleanliness			5.532	0.019
Normal	163 (50.94)	72 (40.00)		
Abnormal	157 (49.06)	108 (60.00)		
Leukocyte esterase			1.468	0.226
Normal	80 (25.00)	54 (30.00)		
Abnormal	240 (75.00)	126 (70.00)		
Neuraminidase			12.90	<0.001
Normal	272 (85.00)	129 (71.67)		
Abnormal	48 (15.00)	51 (28.33)		
Proline aminopeptidase			40.47	<0.001
Normal	265 (98.75)	147 (81.67)		
Abnormal	4 (1.25)	33 (18.33)		
Acetylglucosaminidase			0.015	0.904
Normal	268 (83.75)	150 (83.33)		
Abnormal	52 (16.25)	30 (16.67)		

*Data is express as n (%)

vaginal flora density and grade II-III vaginal flora diversity ($p<0.001$, Tables 5 and 6).

Occurrence of adverse reactions between two groups

As Table 7 shows, no difference was seen in adverse reactions between the two groups ($p>0.05$).

HPV clearance between two groups

Following the three-month treatment period, HPV testing was repeated. The clearance rate of HPV in the observation group (OG) (interferon α-2b + *Lactobacillus*) was significantly higher compared to the control group (CG) (interferon α-2b alone) (Table 8). Specifically, HPV clearance was observed in:

- OG: 68 of 90 cases (75.56%)
- CG: 51 of 90 cases (56.67%)
- $\chi^2 = 7.156$, $P = 0.0076$

This finding strongly suggests that the addition of *Lactobacillus* vaginal capsules

Table 2. Comparison of positive rate of Bacterial vaginosis, Trichomonas vaginitis y Vulvovaginal candidiasis between the two groups.

Index	HPV-negative group (n=320)	HPV-positive group (n=180)	χ^2	*p*
Bacterial vaginosis			5.023	0.025
Positive	30 (9.38)*	29 (16.11)		
Negative	290 (90.62)	151 (83.89)		
Trichomonas vaginitis			0.183	0.669
Positive	15 (4.69)	10 (5.56)		
Negative	305 (95.31)	170 (94.44)		
Vulvovaginal candidiasis			0.020	0.142
Positive	29 (9.06)	17 (9.44)		
Negative	291 (90.94)	163 (90.56)		

*Data is expressed as n (%)

Table 3. Correlation between HPV infection and vaginal microecology.

Influencing factors	β	SE	Wald	OR	95% CI	p
Catalase	1.0	0.1	66.5	2.8	2.2-3.6	<0.05
Proline aminopeptidase	1.7	0.4	15.0	5.6	2.3-13.5	<0.05
Bacterial vaginosis	1.1	0.5	4.5	3.1	1.1-8.9	<0.05

Table 4. Clinical efficacy between the two groups.

Groups	Cases (n)	Obvious Effect n (%)	Effective n (%)	Ineffective n (%)	Total Effective Rate n (%)	χ^2	*p*
Control Group	90	38 (42.23%)*	32 (35.55%)	20 (22.22%)	70 (77.78%)	10.22	0.001
Observation Group	90	57 (63.33%)	28 (31.11%)	5 (5.56%)	85 (94.44%)		

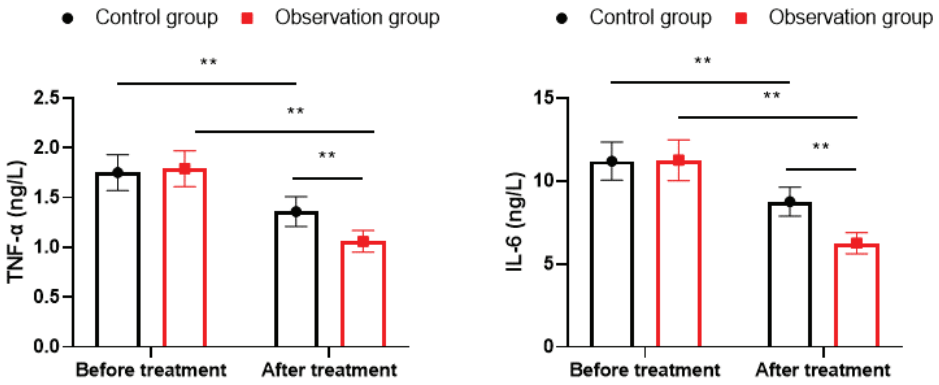

Fig. 1. Comparison of inflammation between the two groups. **p<0.01.

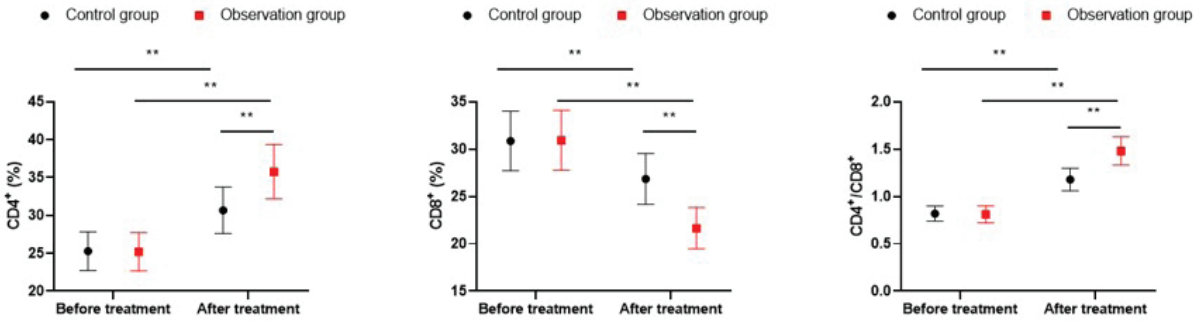

Fig. 2. Immune function between the two groups. **p<0.01.

Table 5. Vaginal flora density (grade II-III).

Groups	Cases	Vaginal flora density				χ^2	p
		Before treatment		After treatment			
		Cases	%	Cases	%		
Control group	90	36	40.00	61	67.78	13.97	<0.001
Observation group	90	34	37.78	81	90.00	53.19	<0.001
χ^2		0.093		13.34			
p		0.759		<0.001			

Table 6. Vaginal flora diversity (grade II-III).

Groups	Cases	Vaginal flora density				χ^2	p
		Before treatment		After treatment			
		Cases	%	Cases	%		
Control group	90	39	43.33	60	66.67	9.899	0.002
Observation group	90	41	45.56	83	92.23	45.73	<0.001
χ^2		0.090		18.00			
p		0.764		<0.001			

Table 7. Occurrence of adverse reactions between two groups.

Groups	Cases	Burning sensation of vulva	Pruritus	Gastrointestinal discomfort	Total incidence rate
Control group	90	2 (2.22)*	0 (0.00)	2 (2.22)	4 (4.44)
Observation group	90	2 (2.22)	1 (1.11)	2 (2.22)	5 (5.55)
χ^2					0.117
p					0.732

*Data is express as n (%).

Table 8. Comparison of HPV clearance between the two groups after treatment.

Group	Total Cases (n)	HPV Negative (Cleared)	HPV Positive (Persistent)	Clearance Rate (%)	χ^2	p
Control Group	90	51	39	56.67%		
Observation Group	90	68	22	75.56%	7.156	0.0076

improves the antiviral effect of interferon α-2b and contributes to a more favorable microecological environment for viral clearance.

DISCUSSION

About 10% to 30% of adults are infected with HPV, and sexually active people are even higher, reaching 50% to 80%, and the most susceptible age is 20 to 29 years old [13]. After HPV infection, some patients continue to be infected with high-risk HPV, cervical intraepithelial tumour-like lesions, after 10 to 15 years, the development of early cervical cancer, and then advanced cervical cancer [14]. HR-HPV is the leading cause of cervical cancer, with 99% of cervical cancer HPV positive, of which HPV16 and HPV18 accounted for about 70%. HPV infection is necessary to cause cervical cancer, but the number of copies of HPV-DNA is not directly related to disease progression, and only persistent high-risk HPV infection increases the risk of cervical cancer [15]. At present, HPV monitoring is the primary screening method for cervical cancer, the tracking method of cervical lesions after treatment and the indicator of the risk of cervical intraepithelial neoplasia [16].

The vaginal microecosystem is a relatively complex system among the four major human ecosystems, which plays an important role in preventing microbial invasion [17]. In our study, the results showed that compared to the HPV-negative group, the HPV-positive group presented a higher abnormal rate of *Lactobacillus*, catalase, cleanliness, neuraminidase, and proline aminopeptidase, consistent with previous reports [18,19]. The typical vaginal microecological environment of women is *Lactobacillus* as the dominant bacteria [20]. *Lactobacillus* can maintain a stable pH value (pH ≤4.5) in the vagina by producing lactic acid, lactobacillin, H_2O_2, and bioactive substances so that the vaginal flora can maintain a balanced state, and through its adsorption on the vaginal mucosa, the formation of a biological barrier against pathogenic microorganisms [21]. Hydrogen peroxide can inhibit or kill bacteria, and the function of catalase is to promote the decomposition of hydrogen peroxide, and reduce the effect of hydrogen peroxide on killing pathogens, resulting in the survival and proliferation of pathogens in the vagina, increasing HPV susceptibility [22]. Reproductive system infections can also lead to changes in cleanliness, abnormal levels of neuraminidase and proline aminopeptidase, aggravate vaginal microecological environment disorders, form a vicious cycle, and increase the risk of HPV infection [23]. In addition, HPV infection may also cause oxidative stress, damage the antioxidant oxidase system in the patient, reduce the level of antioxidants, and damage cellular DNA. In the process of cellular DNA replication, HPV DNA can be transferred to the DNA of host cells, aggravating the degree of mucosal damage, further aggravating the abnormality of the vaginal microecological environment, and forming a vicious cycle [24]. Therefore, the vaginal flora is the first line of defense against pathogenic microorganisms.

Our study also revealed that relative to the HPV-negative group, the HPV-positive group presented a higher positive rate of BV. The reason is as follows: (1) The decrease or disappearance of vaginal *Lactobacillus* in BV patients provides opportunities for the growth and reproduction of other bacteria

such as Gardner-bacteria and anaerobic bacteria, causing the imbalance of vaginal microecological environment, the reduction of the protective function of *Lactobacillus* on the vagina, and the decreased ability of vaginal virus clearance, thus susceptible to HPV infection [25]; (2) BV can affect the expression of vaginal local immune factors, thereby destroying the immune response and making it more susceptible to HPV infection [26]; (3) BV can cause the destruction of cytoskeleton protein of vaginal mucosal epithelium, accelerate the damage of vaginal mucosal epithelial cells, and increase susceptibility to HPV[27]; (4) The sialoglycoidase produced by BV leads to the degradation of vaginal mucosal epithelial protective factors, which enables bacteria to adhere and form biofilms, further leading to the difficulty of HPV removal and the formation of persistent infection [28]. For these reasons, BV not only increases the susceptibility to HPV but also delays the body's clearance of HPV.

Antiviral therapy is the preferred treatment for HPV infection, with commonly used drugs such as recombinant human interferon α-2b gel, an important glycoprotein produced by white blood cells that play a key role in fighting viral infection and has antibacterial activity against a variety of pathogens [29]. Interferon α-2b can inhibit cell proliferation, inhibit angiogenesis, and have cytotoxic effects on tumor cells in various ways [30]. On the one hand, it can bind to specific receptors on the cell surface, thereby inhibiting the growth of virus-infected cells and prolonging the cell cycle of malignant cells [31]. On the other hand, biosynthase plays an important role in promoting cell proliferation in viral cells, and the inhibition of interferon α-2b can play a good role in inhibiting viral replication [32]. In treating HPV infection, transvaginal medication can directly act on cervical epithelial cells and has a specific effect. However, the vagina has a complex microenvironment, which can produce metabolites that compete with drug receptors under the action of the microbiome, resulting in limited effects of single drugs [33].

In this study, the observation group was given *Lactobacillus* vaginal capsules based on interferon α-2b, and the results manifested that relative to the CG, the OG presented a higher total effective rate. The reason may be that *Lactobacillus* vaginal capsule is a kind of microbial preparation, with living intestinal streptococcus as the main component [34], which can increase the production of acidic substances through the decomposition of internal glycogen, thus reducing the pH value of the vaginal environment, effectively promoting the proliferation of *Lactobacillus* in the host body, adjusting the microecological environment, strengthening the self-purification function of the mucosal immune system, and thus enabling the mucosal immune system to rebuild more effectively [35]. In addition, *Lactobacillus* vaginal capsules play a particular role in regulating the immune system, stimulating the release of antibodies in the body and inhibiting the reproduction of bacteria [36]. By rebuilding a good vaginal environment and establishing a protective barrier, the effect of interferon α-2b can be reduced, and the clinical efficacy can be improved.

IL-6 and TNF-α are inflammatory factors that correlate specifically with HPV infection[37]. When the body is infected with HPV, the levels of IL-6 and TNF-α as pro-inflammatory factors will increase abnormally [38]. Therefore, the body's inflammation can be judged by observing the changes of IL-6 and TNF-α levels in the body. The results of our study manifested that relative to the CG, the OG presented lower levels of the above inflammatory markers after therapy, suggesting that *Lactobacillus* vaginal capsules combined with recombinant human interferon α-2b gel could suppress inflammation of the body. Similarly, Dobrohotova et al. suggested that the additional use of *Lactobacillus* vaginal capsules in the complex therapy of lower urinary tract infections reduced inflammation [39].

Under normal circumstances, the body's own immune system can resist the invasion of pathogenic microorganisms, but the immune system has obstacles; the resistance to viruses will decline, easy to cause viral infections, such as HPV infection [40]. The immune function of the body mainly depends on T lymphocytes. $CD4^+$ can coordinate B cells, promote their differentiation and produce antibodies; $CD8^+$ are viral T lymphocytes. When the body's $CD4^+$, $CD8^+$, and $CD4^+/CD8^+$ decline, the body's immune function is disturbed and vulnerable to HPV invasion [41]. Our study demonstrated that relative to the CG, the OG presented better improvements of $CD4^+$, $CD8^+$ and $CD4^+/CD8^+$ levels after therapy, suggesting that *Lactobacillus* vaginal capsule combined with recombinant human interferon α-2b gel could enhance the immune function of the body, which was following a study proposed by Ang et al. [42].

In addition, our study pointed out that relative to the CG, the OG presented a higher proportion of grade II-III vaginal flora density and grade II-III vaginal flora diversity. The reason is that recombinant human interferon α-2b can regulate immune responses by activating intracellular signalling pathways [43]. The drug acts on immune cells, such as macrophages and dendritic cells, thereby enhancing their phagocytosis [44]. Recombinant human interferon α-2b induces a series of cell signalling events by binding to specific receptors, ultimately activating and enhancing immune cells [45]. Besides, HPV infection is directly related to the dysregulation of the vaginal microenvironment and the lack of *Lactobacillus*, which leads to a vicious cycle of the disease [46]. After the application of recombinant human interferon α-2b, the proliferation of antiviral protein in the body was promoted, and the antiviral ability was improved [47]. At the same time, the *lactobacillus* vaginal capsule is vaginally administered, which takes live lactic acid bacteria as the main component and has a certain antibacterial effect, and inhibits the reproduction of harmful bacteria by producing organic acids and other antibacterial substances [48]. In addition, *Lactobacillus* vaginal capsules contain a large number of active lactic acid bacteria cells. After vaginal medication, it can increase the number of beneficial bacteria, inhibit the excessive reproduction of harmful bacteria, promote the balance of vaginal flora and maintain a healthy microecological environment [49].

Moreover, the results of this study showed that there was no statistical difference in the total incidence of adverse reactions between the two groups, which reflected that the adverse reactions produced by *Lactobacillus* vaginal capsule combined with recombinant human interferon α-2b gel were within the acceptable range and the safety was reasonable.

In conclusion, our study indicates that HPV is significantly correlated with vaginal microecological environment, and catalase, proline aminopeptidase and BV were closely related to HPV infection. In addition, the *Lactobacillus* vaginal capsule combined with recombinant human interferon α-2b gel has practical clinical efficacy, which can reduce inflammation, promote immune function, improve vaginal microecological environment, and is safe in the treatment of patients with HPV infection.

ACKNOWLEDGMENTS

None.

Funding

There was no funding for the study.

Conflict of interest

There is no conflict of interest with this manuscript.

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Participation of the authors

YS and LL: conceived and designed the study, as well as collected and analysed the data. WX and CM: drafted and reviewed the manuscript, and finally approved the manuscript.

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