ORIGINAL RESEARCH

Correlation Between Persistent HPV Infection and Vaginal Microecological Imbalance After Treatment of Cervical High-Grade Squamous Intraepithelial Lesion

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ABSTRACT

Objective • To analyze the association between persistent human papillomavirus (HPV) infection and vaginal microecological imbalance after surgical treatment of cervical high-grade squamous intraepithelial lesion (HSIL).

Methods • This is a retrospective study, 180 cervical HSIL patients admitted to our hospital from May 2019 to May 2021 were selected, of these, 84 were treated with loop electrosurgical excision procedure (LEEP) and 96 with cold knife conization (CKC). Patients were followed up for HPV infection 1 year after surgery. There is a division into a persistent infection group (positive group) and a negative group based on the presence or absence of HPV, the detection technique was PCR amplification. The two groups were compared regarding preoperative HPV infection, vaginal micro-ecological indicators 1 year after surgery, and the correlation between persistent HPV infection and vaginal microecological imbalance.

Results • At 1 year after surgery, among 180 cervical HSIL patients, 64 (35.56%) were persistently infected with HPV, with an age of (40.20 ± 4.85) years, including 36 (56.25%) with cervical intraepithelial neoplasia (CIN) grade II, 28 (43.75%) with cervical intraepithelial neoplasia (CIN) grade III, 116 (64.44%) with HPV negative, with an age of (40.22 ± 5.15) years, including 67 (57.76%) with CIN grade II and 49 (42.24%) with CIN grade III, the differences in age and CIN classification between the two groups were not statistically significant ($P > .05$). Preoperatively, 53 people (82.81%) with HPV viral load >100 RLU/CO in the HPV persistent infection group and 76 people (65.52%) with HPV viral load >100 RLU/CO in the HPV negative group, with statistically significant differences between the two groups ($P < .05$); The difference in HPV virus typing and HPV infection type between the two groups was not statistically significant ($P > .05$). At 1 year after surgery, the composition ratio of flora density class IV and flora diversity class IV were significantly higher in the HPV persistent infection group than in the HPV negative group, and the dominant bacteria were mainly gram-positive large bacillus, accounting for 83.33%, the difference between the two groups was statistically significant ($P < .05$); The differences in Nugent scores and pH values between the two groups were not statistically significant ($P > .05$). Logistic regression analysis showed that flora density, flora diversity, and dominant bacteria were all independent risk factors for persistent HPV infection after treatment in patients with HSIL ($P < .05$).

Conclusion • After treatment of HSIL patients, clinical attention should be paid to monitoring of HPV infection but also to the changes in vaginal microecology, as timely correction of vaginal microecology can facilitate HPV regression and improve the patient's prognosis. (*Altern Ther Health Med.* 2024;30(1):414-418).

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INTRODUCTION

The uterine cervix's high-grade squamous intraepithelial lesion (HSIL) is an irreversible precancerous lesion. The histological features are the heterogeneity of cells throughout the layer, human papillomavirus (HPV) infection of the underlying cells of cervical squamous cells leads to accelerated maturation of damaged cells, atypical hyperplasia, atypical keratinization, koilocyte, and other superficial squamous cells are increased.[1] At present, it is generally accepted that about 80% of HPV-infected people can spontaneously resolve through the self-regulation of the body within 2 years after infection, and the patients will not have clinical symptoms or

increased risk of cervical diseases.[2] However, some people show a persistent infection due to various aspects of the virus and the organism. HPV16/18 are the two high-risk HPV subtypes with the highest oncogenic potential.[3] According to statistics, 55%-60% of all cervical cancer cases worldwide are associated with HPV16 infection; 10%-15% with HPV18 infection and the rest with other high-risk HPV subtypes.[4]

HSIL includes cervical intraepithelial neoplasia (CIN) II-III and carcinoma in situ, which, if left untreated, carries a significant risk of progression to invasive cervical cancer. Loop electrosurgical excision procedure (LEEP) and cold knife conization (CKC) are currently the main modalities for the management of HSIL patients with fertility requirements; they can control the postoperative HPV infection rate to around 20.00% and 3.33%, respectively.[5,6] It has been suggested that in addition to the influence of risk factors such as increasing age, polygamous relationships, resection margins, high preoperative viral load, and preoperative HPV E6/E7 mRNA levels, other synergistic factors such as alterations in the vaginal microecological environment may also play an important role in the pathological process of persistent HPV infection after HSIL surgery.[7] Under normal circumstances, the female vaginal microenvironment is in a balanced state. When the abnormal dominant bacteria or pathogens reproduce abnormally, the balance is broken, the vaginal defense mechanism drops, harmful microorganisms constantly invade, and carcinogens and pro-inflammatory factors accelerate the occurrence of carcinogenesis.[8] In recent years, with the advancement of molecular biology and epidemiological studies, the relationship between altered vaginal micro-ecological balance and HPV regression, HSIL occurrence and HSIL prognosis has received clinical attention. In this study, the correlation between persistent HPV infection and vaginal microecological imbalance after surgical HSIL treatment wasanalyzed by microecological assessment of vaginal secretions in 180 HSIL patients with and without HPV infection 1 year after treatment, with the aim of providing new ideas to improve the clinical outcome of HSIL and to improve the prognosis of patients.

MATERIALS AND METHODS

Research Object

This is a retrospective study; a total of 180 patients with cervical HSIL who underwent LEEP or CKC treatment in our hospital from May 2019 to May 2021 and finally completed postoperative follow-up and met the inclusion criteria were selected, of these, 84 were treated with LEEP and 96 with CKC. Patients were divided into the HPV persistent infection group and the HPV negative group based on whether they converted to HPV negative 1 year after treatment.

Inclusion Criteria

The female of childbearing age with a history of sexual intercourse and non-pregnancy; preoperative diagnosis of HSIL confirmed by pathological histology; preoperative confirmed HPV and CIN infection, and targeted HPV treatment was not carried out; no history of sex hormones, vaginal irrigation, and antibiotic treatment within 3 months and no history of immunosuppressive therapy within 6 months; no other malignancies in combination; complete medical records and completion of 1 year postoperative follow-up.

Exclusion Criteria

Total hysterectomy and radiotherapy; pregnant or lactating women; immunocompromised patients with various immune diseases, malignant tumours or immunosuppressive drugs; those with combined severe cardiac, hepatic, renal and haematopoietic diseases and psychiatric disorders.

HPV Test

Before and one year after the operation, the HPV fluorescence detection kit of Guangdong Kaipu Biotechnology company was used to detect HPV infection by PCR amplification technology (ABI7500 automatic gene amplification instrument). A tissue sample from the lesion or the suspected site was taken, DNA was extracted, the target DNA was amplified, color developed using specific primers, and the results were finally determined. High-risk HPV typing: 16/18/31/33/35/39/45/51/52/56/58/59/68. Determination of HPV persistent infection: if a genotype of HPV is positive for more than two consecutive tests, it is a persistent infection.

Vaginal Discharge Test

1 year after surgery, vaginal secretions collected were assessed using the bPR-2014A vaginal microecological evaluation system. Sampling method: exposure of the vagina by means of a vaginal dilator, and 2 long sterile cotton swabs were rotated proximally in the upper 1/3 of the vagina for 10 s; then, the samples were laid flat on 2 slides for testing. Indicators include flora density, flora diversity, dominant bacteria assessment, Nugent score, and pH value. The specific grading criteria for each indicator are shown in Table 1.

Statistical analysis

Statistic Package for Social Science (SPSS) 23.0 software (IBM, Armonk, NY, USA) was applied. Count data were expressed as ratio and measurement data as mean ± standard

Table 1. Grading criteria for each vaginal microecological indicator

Indicators	Classification	Standard
Flora density (Bacterial count under per oil microscope)	Grade I	1~99 bacterias
	Grade II	100~999 bacterias
	Grade III	>1000 bacterias
	Grade IV	Full vision
Flora diversity (bacterial species under oil microscope)	Grade I	1~3 kinds
	Grade II	4~6 kinds
	Grade III	7~9 kinds
	Grade IV	>10 kinds
Dominant bacteria	Gram-negative short bacilli	
	Gram-positive cocci	
	Gram-positive large bacillus	
Nugent score	Normal	0~3 scores
	Middle	4~6 scores
	Severe	>7 scores
pH value	Normal	3.8~4.6
	Abnormal	>6

deviation (M±SD), and the χ^2 test and *t* test were used, respectively. Logistic regression analysis was used for the analysis of influencing factors. Statistical significance was expressed as $P < .05$.

RESULTS

HPV infection 1 year after surgery in 180 patients with HSIL

At 1 year after surgery, among 180 cervical HSIL patients, 64 (35.56%) were persistently infected with HPV, 116 (64.44%) with HPV negative. (Figure 1)

At 1 year after surgery, the mean age of the HPV persistent infection group was (40.20±4.85) years, of which 36 (56.25%) were CIN grade II and 28 (43.75%) were grade III, and the mean age of the HPV negative group was (40.22±5.15) years, of which 67 (57.76%) were CIN grade II and 49 (42.24%) were grade III, the differences in age and CIN classification between the two groups were not statistically significant (according to the statistical results, $P > .05$). (Figure 2)

Preoperative HPV infection in 180 patients with HSIL

Preoperatively, 53 people (82.81%) with HPV viral load >100 RLU/CO in the HPV persistent infection group and 76 people (65.52%) with HPV viral load >100 RLU/CO in the HPV negative group, according to the statistical results, $P < .05$, the difference is statistically significant. Preoperatively, 52 (81.25%) of the HPV persistent infection group had HPV virus typing of 16/18, and 83 (71.55%) of the HPV negative group had HPV virus typing of 16/18, according to the statistical results, $P > .05$, the difference was not statistically significant. Preoperatively, 46 (71.88%) of the HPV persistent infection group had a single type of HPV infection, and 86 (74.14%) of the HPV negative group had a single type of HPV infection, according to the statistical results, $P > .05$, the difference was not statistically significant. (Figure 3)

Vaginal microecological indicators 1 year after surgery in 180 patients with HSIL

At 1 year after surgery, the composition ratio of flora density class IV and bacterial diversity class IV were significantly higher in the HPV persistent infection group than in the HPV negative group, and the dominant bacteria were mainly gram-positive large bacillus, accounting for 83.33%, the difference between the two groups was statistically significant (according to the statistical results, $P < .05$); The differences in Nugent scores and pH values between the two groups were not statistically significant (according to the statistical results, $P > .05$). (Figure 4)

Correlation analysis of persistent HPV infection and vaginal microecological imbalance

Logistic regression analysis showed that flora density, flora diversity, and dominant bacteria were all independent risk factors for persistent HPV infection after treatment in patients with HSIL (according to the statistical results, $P < .05$). (Table 2)

Figure 1. HPV infection 1 year after surgery in 180 patients with HSIL. Age and CIN classification distribution in the HPV positive and negative groups at 1 year postoperatively

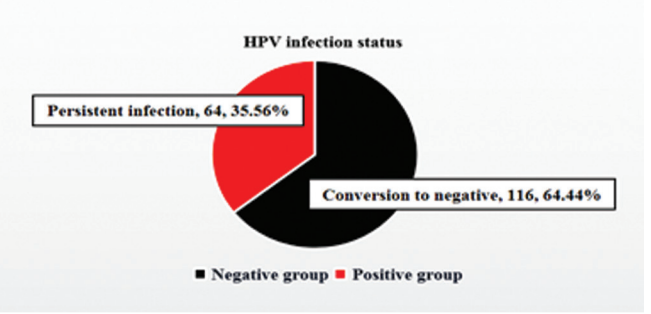


Figure 2. Age and CIN classification distribution in the HPV positive and negative groups at 1 year postoperatively. (A) Age distribution; (B) Distribution of CIN classification.

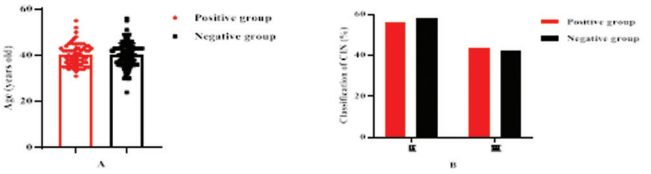
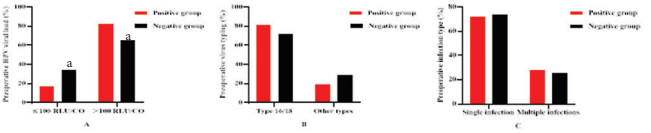
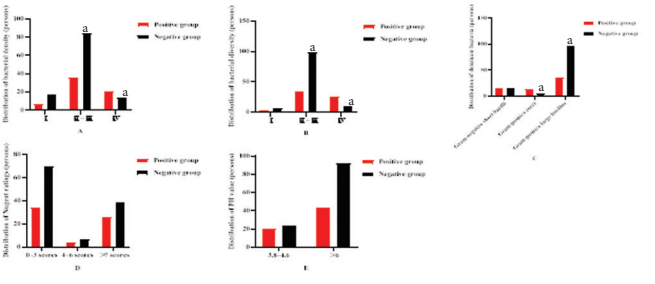


Figure 3. Preoperative HPV infection in the HPV-positive and negative groups. Note: (A) Preoperative HPV viral load status; (B) Preoperative HPV typing status; (C) Preoperative HPV infection type status.



[a]For comparison of similar items between groups, $P < .05$.

Figure 4. Distribution of various vaginal microecological indicators in the HPV positive and negative groups. (A) Distribution of flora density; (B) Distribution of flora diversity; (C) Distribution of dominant bacteria; (D) Distribution of Nugent score; (E) Distribution of pH value.



[a]For comparison of similar items between groups, $P < .05$.

Table 2. Longistic regression analysis of the association between persistent HPV infection and vaginal microecological imbalance

Factors	β	SE	Wald	*P* value	OR	95%CI
Flora density	-1.379	1.258	4.598	.017	0.252	0.021~2.964
Flora diversity	-1.508	1.304	4.123	.030	0.221	0.017~2.851
Dominant bacteria	-1.745	1.523	5.456	.009	0.175	0.009~3.456

DISCUSSION

Currently, the clinical causative factors for the development of HSIL are high-risk HPV infection, which is a double-stranded DNA virus with sexual transmission as the main route of transmission. They can infect host cells in both the integrated and non-integrated state. The persistent infection of HPV suggests a poor prognosis, and the virus is still aggressive to normal tissues, laying a hidden danger for the deterioration of the disease. In addition, the vaginal microenvironment of HSIL patients also had different degrees of disorder. According to statistics, more than 50 species of bacteria are in the vagina, with the dominant bacterium being Lactobacillus. Its balance is closely related to the structural stability of the vaginal flora, local immune response, endocrine metabolism and many other factors.[9] In recent years, it has been suggested that the regression of high-risk HPV infection is related to the balance of vaginal microecology and that improving the environment of vaginal microecology in patients with HSIL can promote recovery from the disease and should be important to clinical workers.[10]

In this study, the microecological assessment of vaginal secretions of 180 patients with HSIL with and without HPV infection 1 year after treatment showed that the incidence of persistent HPV infection in 180 patients with cervical HSIL was 35.56% and 64.44% of patients were converted to negative, with no significant difference in age or CIN classification between the two groups. It is generally accepted that HPV infection is more persistent with increasing age, but different studies have different views on age's defining values. Some foreign studies[11,12] have concluded that women older than 50 years are at significantly increased risk of persistent HPV infection, which may be related to age affecting a woman's own hormonal status. This study is different from the conclusion that the age of some studies can affect the clearance of high-risk HPV, which may be related to the narrow age range and small sample size included in this study.

Many authors[13,14] have suggested that persistent postoperative high-risk HPV infection is positively correlated with preoperative HPV viral load and that high viral load is associated with CIN grade in a dose-effect relationship. However, there is no uniformity in the definition of criteria for distinguishing between high and low viral loads. In the present results, preoperatively, the percentage of HPV viral load >100 RLU/CO in the HPV persistent infection group was 82.81%, which was significantly higher than that of 65.52% in the HPV negative group, which suggests that high preoperative viral load may be an important risk factor for persistent HPV infection in HSIL patients after surgery. HPV subtypes differ in the progression of disease in patients with HSIL. A prospective study in Japan[15] showed that the cumulative progression rate of CIN was significantly higher in HPV 16, 18, 31, 33, 35, 45, 52 and 58 infections than in the other five species (HPV 39, 51, 56, 59 and 58). In this study, the viral typing of HPV infection was dominated by HPV16/18 in both the HPV persistent infection group and the HPV negative group, which again confirms that HPV16/18 has the strongest oncogenic potential. Hong et al.[16] suggested that multiple infections with HPV may have an important role in advancing the pathological development of cervical lesions. Also, Lebelo et al.[17] believe that multiple infections also play a role in HPV persistent infection. Soto[18] and other scholars found in the 24-month follow-up that the negative conversion rate of high-risk HPV multiple infections was higher than that of single infection at various time points after surgery, but there was no statistical difference. In this study, there was no statistically significant difference between the two groups in terms of the type of HPV infection, in partial agreement with the findings of Soto et al.[18], which also suggests that postoperative HSIL patients must be followed up regularly and that if necessary, treated accordingly to avoid progression of the disease.

As a barrier against external viruses and microorganisms, vaginal microecology will produce hydrogen peroxide, defensins, bacteriocin, nutrition, etc., which will affect the colonization and infection of external pathogens. The number of lactobacillus is the key to maintaining the vaginal microecological balance, which can maintain the acidic environment of the vagina and promote the activation of the local immune function of the body.[19] In contrast, when lactobacillus declines, the vaginal environment is altered, and the composition ratio of abnormal diversity (++/+++) and density (++/+++) of the bacterial flora increases, resulting in a relative increase in BV and mixed vaginitis infections.[20] Also, the long-term chronic inflammation of the vagina affects the content and function of lactobacillus, and further weakens the defense function of the microecology, resulting in an increase in the probability of vaginal infection. This repeatedly makes the imbalance of the microecology of the vagina more and more serious, and becomes an opportunity for sustained HPV infection. In this study, the vaginal microecology of the patients was detected one year after the operation, the results showed that the vaginal microecology of the HPV persistent infection group was more disordered than that of the HPV negative group, and the composition ratio of dysbacteriosis was significantly increased, resulting in a relatively high vaginal infection rate; further logistic regression analysis was carried out, the results showed that the flora density, flora diversity, and dominant bacteria were all independent risk factors for persistent HPV infection after treatment in patients with HSIL. It is suggested that the density, diversity, and variety of vaginal microecology after HSIL operation are important for the HPV prognosis of patients; clinical administration of timely and reasonable vaginal microecological agents to regulate the stability of patients' vaginal microecology may promote HPV-negative conversion in patients with HSIL after surgical treatment.

The limitations of this study lie in the specific patient population and the potential challenges of accurately assessing the vaginal microbiota. Incidentally, our next study is planned to compare the differences in vaginal microecological indicators and HPV viral load among

patients with cervical precancer and to analyze the correlation between vaginal microecology and HPV viral load and cervical precancer using Spearman's correlation test in the hope of providing relevant guidance for early screening and prevention of cervical disease.

CONCLUSION

After the treatment of HSIL patients, clinical attention should be paid to monitoring of HPV infection but also to the changes in vaginal microecology, as timely correction of vaginal microecology can facilitate HPV regression and improve the patient's prognosis.

DATA AVAILABILITY STATEMENT

The datas supporting this study's findings are available from the associated author upon reasonable.

CONFLICT OF INTEREST

We do not have any possible conflicts of interest.

ETHICS STATEMENT

Approved by the Hospital Ethics Committee.

AUTHORS CONTRIBUTIONS

SZ and HL designed the study and performed the experiments, YC and YH collected the data, YC, YH and GL analyzed the data, SZ and HL prepared the manuscript. All authors read and approved the final manuscript.

SCRIENTIFIC RESEARCH PROJECT

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