Human Papillomavirus Infection in Relation to Vaginal Microflora and Immune Factors

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Abstract: **Objective:** Clarify the vaginal microflora and immune factors in women with human papilloma virus (HPV) infection, and explore its association with HPV infection. **Methods:** This study collected vaginal secretions and blood from 160 women initially diagnosed as HPV positive in our hospital from June 2020 to December 2020 and 80 healthy women with HPV negative physical examination in the same period. The vaginal microflora of the patients were detected by 16S rDNA sequencing and the expression of immune factors was measured by a high-performance liquid phase chip. **Results:** The different types of HPV were HPV mix (64,40%), HPV52 (39,24.375%), HPV16 (30,18.750%), HPV58 (18,11.250%), HPV18 (6,3.750%), HPV53 (1,0.625%), HPV55 (1,0.625%), and HPV68 (1,0.625%).α diversity analysis showed that there was no significant difference in vaginal microflora between different HPV types (P=0.733). The genus level abundance of vaginal microflora in each group was mainly *Lactobacillus*, followed by *Gardnerella* and *Prevotella*. LEfSe Analysis showed that the mix group was *Gardnerella* and the type HPV16 group was *Streptococcus*. The immune comparison showed that MIP-1β was significantly upregulated in the HPV-positive group, but EGF in the HPV-negative group. **Conclusion:** This study revealed that HPV infection can change the proportion of vaginal microbial bacteria and the expression of immune factors, which provides a basis for local vaginal treatment and prevention of HPV infection after HPV infection.

Keywords: HPV; Vaginal Microflora; Immune Factors; Cervical Cancer

1. Introduction

High-risk human papilloma virus (HR-HPV) infection is the leading cause of cervical cancer, the most common reproductive tract malignancy in women[1]. The most common type of HPV infection in cervical cancer was HPV16, followed by HPV18[2]. The vast majority of HPV infected women can turn negative through autoimmune response, and only a few of those with persistent high-risk HPV can progress to cervical cancer. The vaginal environment consists of local immunity, vaginal microflora and human endocrine regulation[3], which is in a dynamic and balanced state. When the balance is disrupted, it will increase the chance of HPV infection, promoting the development of HPV infection to cervical cancer with possible[4]. The main component of the vaginal microecology of healthy women of reproductive age period is *Lactobacillus*, which is involved in maintaining the weak acidic environment and maintaining the stability of the vaginal microecology, and is considered to be the first line of defense against pathogens[5]. When the balance is broken, the number of lactic acid bacteria is reduced or the function is inhibited, vaginal *Gardnerella* or mixed anaerobic bacteria multiply, produce many harmful metabolites, and then accelerate the occurrence and development of cervical lesions and cervical cancer[6-7]. Studies have shown that immune factors participate in the immune response of the body after HPV infecting the body[8]. When the body is infected by HR-HPV, it can induce the body to produce immune regulatory cells, jointly complete the immune regulation function of the body, and affect the occurrence and development of lesions and tumors. The purpose

of this study is the need to explore the relationship between vaginal microflora and immune factors and HPV infection, so as to provide a basis for the prevention of HPV infection and local vaginal treatment after infection.

2. Materials and Methods

2.1 Study subjects

Women who received or physical examination in our hospital from June 2020 to December 2020 were collected as the study subjects. The inclusion criteria were: Women aged 20 to 60 years with sexual history, No sex life and no vaginal treatment within 72h, informed consent, reviewed and approved by the hospital ethics committee. Exclusion criteria: ongoing vaginal or cervical disease, combined immune disease or other medical disease.

2.2 Detection methods

2.2.1 Vaginal microbiological test:

Secretions were collected with a sterile cotton swab on 1/3 of the patient's vagina and stored in a -80℃ refrigerator. Samples were collected and subjected to 16S rDNA sequencing.

2.2.2 Immune factor detection:

5 mL of peripheral blood was extracted from 5mL blood vessels without anticoagulant, and the serum was isolated, and the immune factors were detected by high-performance liquid phase chip.

2.3 Statistical methods

Microbiological analysis was performed using Kruskal-Wallis H test. The expression of immune factors in patients with different groups was analyzed by using independent samples t-test. $P<0.05$ was considered as a statistically significant difference.

3. Results

Depending on the HPV classification, 160 HPV positive patients were divided into 8 groups, types 16,18,52,53,55,58,68 and mix, respectively. The infection rates of different types of HPV were HPV mix (64,40%), HPV52 (39,24.375%), HPV16 (30,18.750%), HPV58 (18,11.250%), HPV18 (6,3.750%), HPV53 (1,0.625%), HPV55 (1,0.625%), and HPV68 (1,0.625%, Table 1). α Diversity is used to measure the diversity of the microflora in the sample, and we found that there was no significant difference in the vaginal microflora in each group (Figure 1, $P=0.733$).

Table 1: Distribution of the different HPV types

	HPV type(n=160)							
	16	18	52	53	55	58	68	mix
positive	30	6	39	1	1	18	1	64
	18.750%	3.750%	24.375%	0.625%	0.625%	11.250%	0.625%	40%

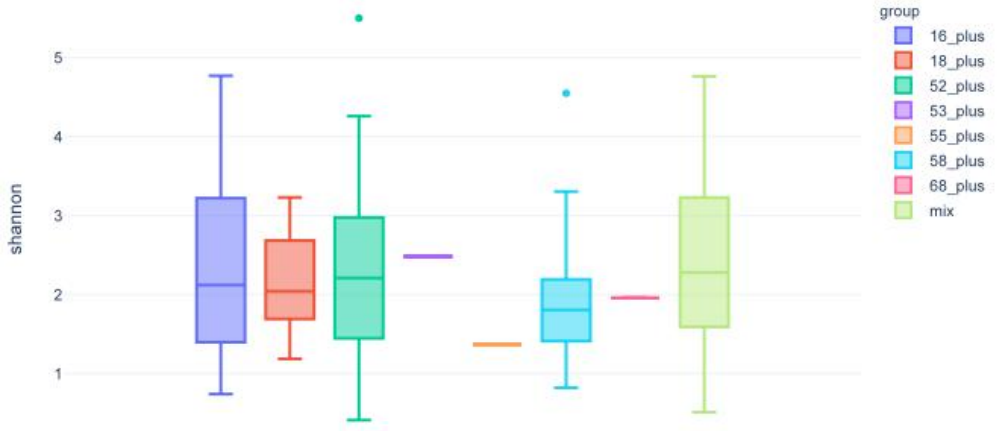

Figure1 The α-diversity of vaginal microbiota

To further analyze the similarities and differences of each group, we analyzed species relative abundance in each group. It can be clearly seen from the relative abundance bar chart that the genus level abundance of the vaginal microflora was dominated by lactobacillus, followed by *Gardnerella* and *Prevotella* (Figure 2).

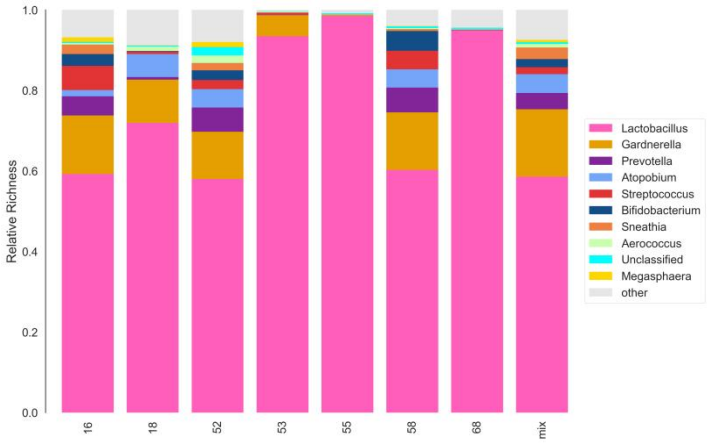

Figure2 Bar chart of the relative abundance of genus-horizontal species

In order to identify the significantly different microflora in each group, the effect size based on linear discriminant analysis (LEfSe analysis), and the microflora with LDA> 4 were used. Among them, the mix group was *Gardnerella* (Figure3, P <0.05), and the type HPV16 group was *Streptococcus* (Figure4, P <0.05). The remaining groups found no differential bacteria at the level of LDA> 4.

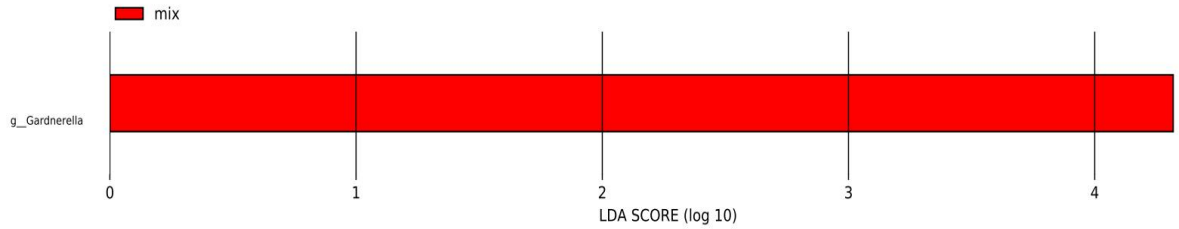

Figure3：LEfSe histogram of mix group (LDA> 4)

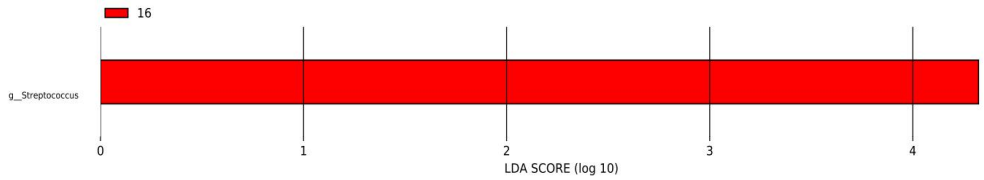

Figure 4 LEfSe histogramof HPV 16 group (LDA> 4)

To explore the relationship between HPV infection and immune factors, we compared and analyzed the differences in the expression of immune factors between HPV-positive and HPV-negative patients. Serserum of 80 HPV-positive patients were mixed into a new sample and divided into 4 groups (named C1, C2, C3 and C4), and 80 HPV-negative healthy women per 20 mixed samples (named D1, D2, D3 and D4) were analyzed for comprehensive analysis. The results showed that MIP-1β was significantly increased in the HPV positive group (C group), while EGF was significantly increased in the HPV-negative group (D group)(Figure5, $P<0.05$).

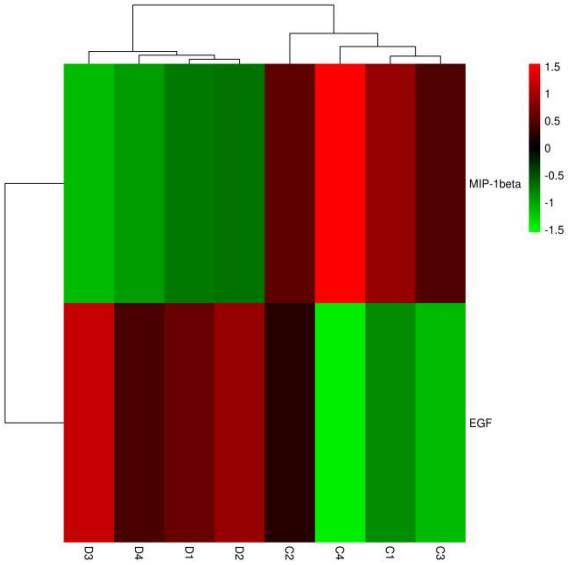

Figure 5 Heat map of the immune factors

4. Discussion

Under normal circumstances, the vaginal environment of women is acidic, and the dominant microflora and other bacteria are in a dynamic balance state, which can effectively prevent HPV infection. When this acidic environment is destroyed, the structure of the microflora will change [9-10]. Continuous HR-HPV infection is a key factor in the progression of cervical epithelial cells to cervical lesions and even to undergo malignant transformation [11]. Among them, HPV16/18 are the two most common high-risk types of HPV leading to cervical lesions[12]. Different types of HPV infection can lead to cervical cancer,with types 16,18, 31, 33, 39, 45, 51, 52, 56, 58, 59, 66 and 68 considered high-risk [13]. In our study, the top 5 most common HPV genotypes were HPV mix, HPV52, HPV16, HPV58 and HPV18. According to this study, the differential bacteria in mix group were *Gardnerella*, and the differential bacteria in type HPV16 group were *Streptococcus*. Therefore, it is speculated that *Gardnerella* and *Streptococcus* may be synergistic factors for HPV infection. In future clinical work, patients with types HPV mix and HPV16 should focus on the detection of microflora *Gardnerella* and *Streptococcus*.

Comparison of immune factors between the HPV-positive and HPV-negative groups revealed that MIP-1β was significantly upregulated in the HPV-positive group, whereas EGF was significantly upregulated in the HPV-negative

group.The results of this study showed that the expression of EGF in the HPV positive group was significantly reduced compared with the HPV negative group, so the reason for the decrease in EGF may be due to the massive replication of the virus, which led to the increased EGF consumption. It is speculated that EGF may play an important immune defense function. However, MIP-1β expression was significantly upregulated when HPV was positive, and it was speculated that the expression of MIP-1β might be increased by HPV infection.

5. Conclusion

In conclusion, this study showed that HPV infection could cause changes in vaginal microflora and body immune factors. When HPV infection, immune control is weakened and the expression of immune factor EGF is reduced. MIP-1β was increased after HPV infection. It is speculated that EGF may play an important immune defense function, and the microflora *Gardnerella* and *Streptococcus* may be the synergistic factors of HPV infection.

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