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Original Research

Correlation between Indicators of Vaginal Microbiota and Human Papillomavirus Infection: A Retrospective Study

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Abstract

Background: Increasing evidence suggests that both the vaginal microbiota and human papillomavirus (HPV) may play a role in the development of cervical intraepithelial neoplasia (CIN) and cervical cancer. However, the relationship between the vaginal microbiome and HPV infection remains poorly understood. The objective of this study was to investigate the association between indicators of the vaginal microbiota and HPV infection. **Methods**: From January 2020 to June 2022, clinical data were collected from 5099 outpatients at Beijing Friendship Hospital. These patients underwent simultaneous testing for vaginal microecology and HPV type. A statistical analysis was conducted to examine the relationship between indicators of the vaginal microbiota and HPV infection. **Results**: HPV infections were detected in 12.47% (636/5099) of the subjects. Single, double, triple, quadruple, and quintuple infections accounted for 81.29%, 14.62%, 3.14%, 0.94%, and 0.15% of all infections, respectively. A significant disparity in HPV infection prevalence was observed between the vaginitis group and the general population. However, no variation was found among different vaginitis groups. The data indicated that individuals with clue cells and sialidase were more susceptible to HPV infection. Sialidase was identified as an independent risk factor for HPV infection in a multivariable logistic regression model. The most prevalent HPV subtypes were 16 and 52, representing 2.10% and 2.86%, 3.14% and 2.86%, 1.78% and 2.16% in the normal, bacterial vaginitis, and other groups, respectively. **Conclusions**: Our findings demonstrate the presence of clue cells and sialidase, which are two diagnostic criteria for bacterial vaginitis, in association with HPV infection. Furthermore, our results suggest that sialidase could potentially serve as a valuable predictor of HPV infection.

Keywords: vaginal microbiota; HPV infection; retrospective study

1. Introduction

The vaginal microbiota is a complex and self-regulating system. *Lactobacillus* species, particularly *Lactobacillus* app., dominate the vaginal microbiota and play a crucial role in maintaining its balance [1]. These bacteria produce lactic acid and other bactericidal chemicals through the digestion of epithelial cells, effectively inhibiting the growth and reproduction of other microbial species. Within the vaginal microbiota, different strains of *Lactobacillus* compete with compete with each other to maintain a delicate equilibrium. Disruption of this balance can lead to microbiota disorders. Microbiota disorder will arise if this balance is disrupted. A healthy vaginal microbiome is essential for protecting reproductive health and preventing sexually transmitted infections. Conversely, an imbalance in the vaginal microbiota can increase the risk of various complications, including intraepithelial lesions and even cervical malignancy [2,3].

Papillomavirus, a double-stranded DNA virus, is capable of causing infections in mucosal epithelial tissues in various areas of the body, including the vulva, vagina, anus, penis, and head and neck [4,5]. The prevalence of human papillomavirus (HPV) infection is high, with over 200 different types of HPV classified as either high-risk type (HR-HPV) or low-risk type (LR-HPV) based on their carcinogenic potential [6,7]. Given the similarities in the effects and infection sites of microbiota diseases and HPV, there may be potential connections between them. However, it remains unclear whether these connections exist and which factor may have been the initial cause.

In this retrospective study, we collected data from a large cohort of subjects who had undergone tests for vaginal microecology and HPV types. The objective was to assess the association between vaginal microbiota and HPV infection. Previous studies have primarily focused on the relationship between vaginal microbiota and HR-HPV, while neglecting LR-HPV and microbial composition in a limited sample, using 16S rDNA technology [8–12]. In contrast, our study examined both HR-HPV and LR-HPV, providing a comprehensive understanding of their association. Furthermore, a thorough examination of microecological parameters and HPV typing will contribute to local epidemiological investigations and future research endeavors.

2. Materials and Methods

2.1 Study Population

Clinical data were collected from January 2020 to June 2022 at Beijing Friendship Hospital, Capital Medical University, a 2300-bed general ward. The data was obtained through the Laboratory Information Management System (LIS). The study comprised 5099 participants from the outpatient department who underwent concurrent testing for vaginal microecology and HPV typing. All subjects met the following inclusion criteria: (i) absence of medication (e.g., antibiotics or hormones) or undergone invasive cervical inspections; (ii) no vaginal lavage or sexual intercourse within 48 hours prior to sample collection; (iii) absence of sexually transmitted diseases. This study was approved by the institutional review boards of Beijing Friendship Hospital, Capital Medical University. The informed consent was obtained from legal guardians for participants under 18 years of age. All experiments were conducted in accordance with applicable laws, institutional guidelines, and the ethical standards outlined in the Declaration of Helsinki.

2.2 Sample Collection

Sterile cotton swabs were utilized for the collection of vaginal microecology samples. The swabs were gently inserted into the upper third of the vaginal wall, and rotated a duration of 10–15 seconds. Subsequently, the collected samples were evenly distributed onto a clean slide. Additionally, another sterile cotton swab was employed to collect secretions from the same location. These samples were stored at a temperature of –20 °C to facilitate subsequent analysis.

Cervical exfoliated cells for cervical HPV specimens were obtained using plastic brushes. The brushes were rotated in a clockwise direction five times and the collected cells were subsequently transferred to a sterile tube containing a cell preservation solution. These tubes were then stored at a temperature of –20 °C until DNA extraction.

2.3 Vaginal Microecology

Morphological and enzymatic examinations were performed using the Vaginitis Automatic Detection Workstation (Jiangsu Shuoshi Biotechnology Co., Ltd., Taizhou, Jiangsu, China), following the provided kit instructions. The results of the vaginal microecology analysis were determined based on the Expert Consensus on Clinical Application of Vaginal Microecology Evaluation released in 2016 [13]. A normal vaginal microecology was defined as having a class II–III microbiome density, class II–III microbiome diversity, *Lactobacillus* app. as the dominant bacteria, a pH level of 3.8–4.5, and negative leucocyte esterase indicating the absence of H_2O_2 secretion. Abnormal vaginal microbiota was identified through morphological and functional tests, and categorized as bacterial vaginitis (BV), aerobic vaginitis (AV), vulvovaginal candidiasis (VVC), trichomonas vaginitis (TC), or abnormal flora and flora suppression. Each specimen was tested twice by two experienced technicians, and any uncertainties were resolved by a senior technician.

2.4 DNA Extraction and HPV DNA Genotype Testing

DNA extraction from exfoliated cells and HPV DNA genotype testing were performed using a commercially available kit (20173404697, Shanghai Tellgen Diagnostic Technology Co., Ltd., Shanghai, China) according to the manufacturer's instructions. The cell-containing preservation solution was transferred into a 1.5 mL centrifuge tube after mixing and centrifuged at 14,000 rpm for three minutes. The supernatant was discarded, and the pellet was resuspended in 200 µL of nucleic acid reagent. The mixture was then heated in a 100 °C metal bath for 15 minutes, followed by centrifugation at 14,000 rpm for five minutes. The supernatant containing HPV DNA was processed using multiple polymerase chain reaction (PCR) amplification, microsphere hybridization, and phycoerythrin binding. Finally, the fluorescence signal of the classified microsphere was detected using the flow lattice instrument Luminex200 (20173401166, Luminex Corporation, Austin, TX, USA).

The amplification procedure consisted of an initial cycle at 95 °C for five minutes, followed by 40 cycles of denaturation at 93 °C for 30 seconds, annealing at 58 °C for 30 seconds, and extension at 72 °C for 30 seconds. A extension final cycle at 72 °C for three minutes was performed. Quality control measures were implemented throughout the experiments, encompassing DNA extraction and amplification, utilizing both positive and negative controls.

The method enabled the detection of 20 HPV nucleic acid subtypes, including high-risk HPV subtypes 16, 18, 31, 33, 35, 39, 45, 51, 52, and 56, as well as low-risk HPV subtypes 6, 11, 40, 42, 43, 44, 55, 61, 81, and 83.

2.5 Statistical Analysis

Bivariate associations between indicators of the vaginal microbiota and HPV infection were assessed using the Chi-square test or Fisher exact test for categorical data. Potential covariates with p-values ≤ 0.20 in the univariate analysis were entered into the multivariate regression analysis model via an enter method. Model fit was assessed through the Hosmer–Lemeshow goodness of fit test, with non-significant result indicating adequate fit. Statistical analysis was performed using SPSS Statistics, IBM SPSS Software, v.25.0 (SPSS, Inc, Chicago, IL, USA) and figures were generated using GraphPad Prism 5.0 (GraphPad Software Inc., San Diego, CA, USA). All tests were two-sided, and a significance level of $p \leq 0.05$ were considered statistically significant.

3. Results

In this study, HPV-positive infections were detected in 12.47% (636/5099) of the participants. The prevalence of single, double, triple, quadruple and quintu-

ple infections was found to be 81.29%, 14.62%, 3.14%, 0.94%, and 0.15%, respectively. Among the 20 genotypes analyzed, all except HPV40 (0 cases) were identified (Fig. 1). The three most prevalent HR-HPV types were HPV16 (15.05%, 118/784), HPV52 (13.65%, 107/784), and HPV56 (10.33%, 81/784), while the three most prevalent three LR-HPV types were HPV61 (9.82%, 77/784), HPV6 (7.14%, 56/784), and HPV81(4.97%, 39/784). The frequency of HR-HPV was higher than that of of LR-HPV.

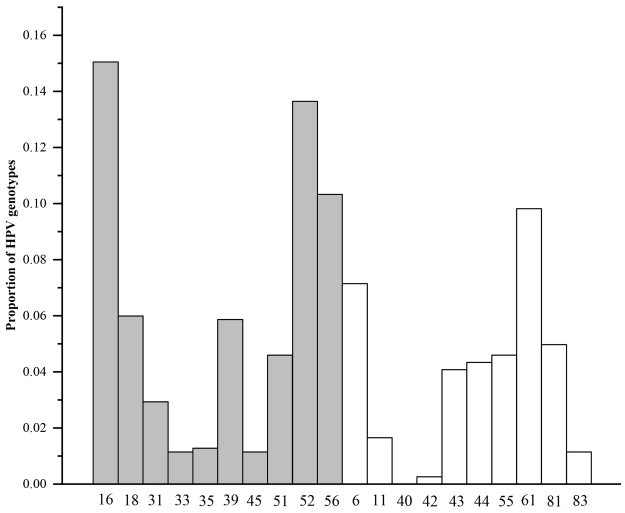

Fig. 1. Distribution of HPV genotypes detected in the participants. High-risk HPV (HR-HPV) were in grey color while low-risk HPV (LR-HPV) in white color. Multiple infections were counted several times. HPV, human papillomavirus.

Table 1 presents the age distribution of the participants and the indicators of vaginal microbiota. A total of 5099 female outpatients were enrolled in the study, with a median age of 35 years (range 14–89 years) at the time of sample collection. The infection rate was slightly higher in the high age group (>45 years) and low age group (<30 years) than that of the middle age group (30–45 years), although no statistically significant differences were observed ($\chi^2 = 5.058$, $p = 0.080$). A significant difference was found in the proportion of HPV between the vaginitis group and the normal population ($\chi^2 = 7.619$, $p = 0.006$). However, no significant differences were identified among different types of vaginitis ($\chi^2 = 9.250$, $p = 0.089$).

The associations of clinical parameters of vaginal microbiota with HPV infection. To further investigate the association between vaginitis and HPV infection, we compared the clinical parameters of vaginal microbiota in relation to HPV prevalence (Table 2). The Chi-square test disclosed that the HPV infection was more frequently observed in participants with clue cells ($\chi^2 = 5.944$, $p = 0.015$) and sialidase positivity ($\chi^2 = 18.338$, $p < 0.001$). These two indicators are related to the diagnosis of BV. However, other clinical parameters of vaginal microbiota including microbiome density, microbiome diversity, classification, Nugent score, leukocyte/oil, pH, leukocyte esterase, catalase, acetyl glycosaminidase, β-glucuronidase showed no difference between HPV positivity and HPV negativity ($p > 0.05$).

Candidate variables associated with an HPV infection with $p \leq 0.2$ were included in the multivariable logistic regression model. These variables included age (odds ratio (OR): 1.005, 95% confidence interval (95% CI): 0.997–1.013, $p = 0.200$), microbiome diversity (OR: 1.209, 95% CI: 0.995–1.469, $p = 0.001$), Nugent score (<4, $p = 0.202$; 4–6, OR: 1.051, 95% CI: 0.849–1.300, $p = 0.649$; ≥7, OR: 1.292, 95% CI: 0.974–1.712, $p = 0.075$), clue cells (OR: 1.429, 95% CI: 1.071–1.906, $p = 0.015$), catalase (OR: 0.822, 95% CI: 0.622–1,087, $p = 0.169$), and sialidase (OR: 2.007, 95% CI: 1.450–2.776, $p < 0.001$). The logistic regression analysis (Table 3) revealed that sialidase significantly increased the risk of HPV infection (OR: 1.982, 95% CI: 1.316–2.987, $p = 0.001$). The Hosmer-Lemeshow goodness-of-fit test indicated a satisfactory overall model fit, with a p value of 0.191.

Distribution of HPV subtypes among the BV group, other group, and normal group. Since sialidase is a specific indicator for BV, we conducted further investigations to examine further explore the difference in the distribution of different subtypes of HPV among the BV group, other group, and normal group (Fig. 2). The most prevalent HPV subtypes were HPV16 and HPV52, accounting for 2.10% and 2.86% in the normal group, 3.14% and 2.86% in the BV group, and 1.78% and 2.16% in the other group, respectively. In contrast, the remaining HPV subtypes accounted for less than 1.70%, except for HPV56 in the Normal group (2.21%) and HPV61 in the BV group (2.05%).

4. Discussion

Recent advancements in gene detection have enabled researchers to focus on genomic investigation of vaginal microbiota in various clinical situations. However, there have been limited studies examining its fundamental properties from a macroscopic perspective. This study aimed to investigate the association between vaginal microbiome and HPV infection using a large cross-sectional survey. The overall HPV positive rate in the target population was 12.47%, slightly lower than the rates reported in other regions of China during the same time period [10,14–17]. Notably, the incidence of HR-HPV types was significantly higher than that of LR-HPV types. Consistent with previous research, HPV16, HPV52, and HPV58 were the most frequently detected types, although their rankings varied. Unfortunately, due to an upgraded Laboratory Information Management System (LIS), there was a lack of relevant information regarding HPV58. The prevalence of HPV16 in cervical cancer exceeded 60% [18]. A recent meta-analysis of eight studies in China also identified HPV16, HPV52, and HPV58 as the most prevalent types in cervical intraep-

Table 1. Age distribution and vaginal microbiota with their association with HPV infection.

Characteristic	Group	HPV-P	HPV-N	Chi-squared analysis	
				χ^2	p
Age (years) n (%)	<30	128 (13.0)	860 (87.0)	5.058	0.080
	30–45	364 (11.7)	2743 (88.3)		
	>45	144 (14.3)	860 (85.7)		
Normal vaginal microbiota or not[a] n (%)	No	340 (13.8)	2124 (86.2)	7.619	0.006
	Yes	296 (11.2)	2337 (88.8)		

a: the dominant bacteria group of normal people is *Lactobacillus*, which has the same meaning with 'Normal vaginal microbiota or not' in this study, so it was not included in the table. HPV-P, HPV-positive population; HPV-N, HPV-negative population; HPV, human papillomavirus.

Table 2. Associations between the indicators of vaginal microbiota and HPV infection.

Characteristic	Group	HPV-P	HPV-N	Chi-squared analysis	
				χ^2	p
Types[a] of abnormal vaginal microbiota n (%)	BV	104 (14.7)	607 (85.4)	8.070	0.138*
	AV	24 (12.3)	171 (87.7)		
	VVC	24 (9.0)	242 (91.0)		
	TC	1 (20.0)	4 (80.0)		
	Abnormal flora	106 (15.2)	592 (84.6)		
	Flora suppression	85 (13.2)	561 (86.8)		
Microbiome density n (%)	Abnormal	155 (14.2)	939 (85.8)	3.666	0.056
	Normal	481 (12.0)	3524 (88.0)		
Microbiome diversity n (%)	Abnormal	128 (13.2)	845 (86.8)	0.513	0.474
	Normal	508 (12.3)	3618 (87.7)		
Classification n (%)	I, II	465 (12.3)	3302 (87.7)	0.220	0.639
	III, IV	171 (12.8)	1161 (87.2)		
Nugent score n (%)	<4	447 (12.1)	3242 (87.9)	3.209	0.201
	4–6	124 (12.7)	865 (87.3)		
	≥7	65 (15.1)	365 (84.9)		
Clue cells	P	61 (16.5)	309 (83.5)	5.944	0.015
	N	574 (12.1)	4154 (87.0)		
Leukocyte/oil	<10	535 (12.4)	3790 (87.6)	0.277	0.598
	>10	101 (13.0)	673 (87.0)		
pH n (%)	3.8–4.5	176 (12.3)	1254 (87.7)	0.050	0.823
	>4.5	460 (12.5)	3209 (87.5)		
Leukocyte esterase n (%)	P	173 (12.7)	1186 (87.3)	0.112	0.738
	N	463 (12.4)	3277 (87.6)		
Catalase n (%)	P	572 (12.3)	4087 (87.7)	1.894	0.169
	N	64 (14.5)	376 (85.5)		
Sialidase n (%)	P	50 (21.6)	182 (78.4)	18.338	<0.001
	N	586 (12.0)	4280 (88.0)		
Acetyl glucosaminidase n (%)	P	74 (13.4)	478 (86.8)	0.488	0.485
	N	562 (12.4)	3983 (87.6)		
β-glucuronidase n (%)	P	10 (14.1)	61 (85.9)	0.171	0.679
	N	626 (12.5)	4402 (87.5)		

Abbreviations: P, positive; N, negative; BV, bacterial vaginitis; AV, aerobic vaginitis; VVC, vulvovaginal candidiasis; TC, trichomonas vaginitis. Complex type: BV+AV, 30 cases; BV+VVC, 21 cases; BV+TC, 1 case; BV+AV+VVC, 1 case; AV+VVC, 2 cases. BV: 14.6% *vs.* Other 13.3%. *Fisher's exact test. a: multiple infections were counted several times.

Table 3. Logistic regression of variables Associations between clinical parameters of vaginal microbiota and HPV infection.

Variable	Univariate logistic regression			Multivariate logistic regression		
	B	OR (95% CI)	*p*	B	OR (95% CI)	*p*
Age	0.005	1.005 (0.997–1.013)	0.200	0.004	1.004 (0.995–1.012)	0.374
Microbiome density	0.190	1.209 (0.995–1.469)	0.056	0.109	1.115 (0.883–1.409)	0.358
Clue cells	0.357	1.429 (1.071–1.906)	0.015	0.502	1.652 (0.837–3.260)	0.148
Catalase	–0.196	0.822 (0.622–1.087)	0.169	–0.102	0.903 (0.678–1.204)	0.487
Nugent score (<4)			0.202			0.286
Nugent score (4–6)	0.049	1.051 (0.849–1.300)	0.649	–0.076	0.927 (0.725–1.184)	0.542
Nugent score (≥7)	0.256	1.292 (0.974–1.712)	0.075	–0.539	0.584 (0.295–1.153)	0.121
Sialidase	0.696	2.007 (1.450–2.776)	<0.001	0.684	1.982 (1.316–2.987)	0.001

Abbreviations: OR, odds ratio; 95% CI, 95% confidence interval; B, regression coefficient.

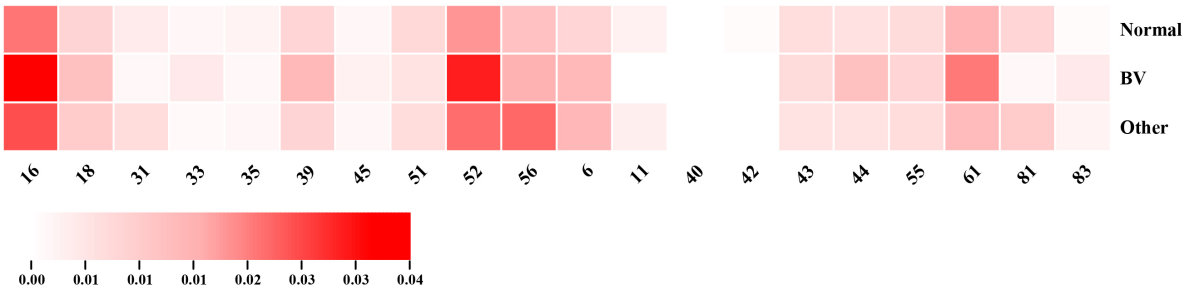

Fig. 2. Distribution of HPV subtypes among the normal group, BV group, and other group. BV group, patients who suffer from BV; Other group, patients who suffer from other vaginitis except for BV; normal group, patients without vaginitis.

ithelial neoplasia (CIN) [15]. These findings suggest that the conventional two-component vaccine may not be effective in preventing cervical cancer, and it is recommended to promote the use of the nine-component vaccine in China. In contrast, LR-HPV types have received less attention in simultaneous research due to their lower frequency and less severe disease-causing potential compared to HR-HPV types. Our findings indicate that HPV61 and HPV6 are the two most prevalent LR-HPV types. Regional distribution was found to influence the prevalence of LR-HPV types, an aspect that has been understudied in previous investigations. While HPV6 and HPV11 are the primary culprits behind condyloma acuminatum and low-grade cervical intraepithelial lesions [19], it is important to also consider LR-HPV types.

According to our research, HPV infection primarily occurs in two age groups: individuals under 30 years old and those over 45 years old, which is consistent with prior studies [20]. The initial increase in HPV infection may be attributed to increased sexual activity or inadequate immunity to HPV, while the subsequent rise in HPV infection could be linked to menopause [21]. Considering the significant impact of age on HPV infection, it is necessary to consider and account for these associations when accurately assessing the clinical parameters of vaginal microbiota related to HPV infection.

PCR validation is essential for the accurate detection of HPV in routine testing. On the other hand, the assessment of vaginal microecology is a complex process that requires skilled clinicians to perform morphological and enzymatic examinations. These examinations involve the evaluation of various signs or scores to determine the presence of conditions such as BV, AV, VVC, TC, aberrant flora, and flora suppression. Investigating the relationship between clinical indicators of vaginal microbiota and an HPV infection would contribute to our understanding of their interplay. Our research findings indicate that outpatients who exhibit clue cells and sialidase positive in their vaginal microbiome have a higher prevalence of HPV infection. Furthermore, additional multivariable logistic regression analysis revealed that sialidase may potentially act as an independent trigger for HPV infection.

Clue cells are a crucial criterion in the traditional Amsel method for the diagnosis of BV [22]. The sialidase, a specific enzyme produced by anaerobic bacteria in the vaginal flora of BV patients, also contributes to BV diagnosis [23]. Although other characteristics, such as the Nugent score, showed no difference among different hierarchies, we assume that the occurrence and development of BV and HPV are related. However, our study found no correlation between types of abnormal vaginal microbiota and HPV infection. The indiscriminate results may be attributed to our rigid grouping and insufficient sample size.

Previous studies suggest that BV could be an induction factor for HPV [24–27]. The mechanism by which BV influences HPV remains undetermined [28]. The main challenge lies in the establishing appropriate animal and cell culture models [29]. BV, characterized by diverse flora, disrupts the mucosal barrier through oxidative stress, inflammatory response, and cytokines expression, potentially leading to the occurrence of sexually transmitted diseases, including HPV infection. Sialidases, which cleave alpha-ketosidic bonds of glycosyl residues, may serve as virulence factors in this process [23,30]. However, one study found that HPV infection occurred earlier or simultaneously with BV [31]. A recent study using the K14-HPV16 transgenic mouse model and examining the NF-κB and Wnt/β-catenin signaling pathways, demonstrated that HPV infection can alter the vaginal microbiome by inhibiting host defense peptides [32]. These findings indicate that the initiating factor remains uncertain apart from their correlation. Further studies are needed to determine the causal relationship between BV and HPV infection.

Furthermore, individuals with BV exhibit a higher prevalence of HPV16 and HPV52 compared to those with normal vaginal microbiota or other abnormal vaginal microbiota. This finding further supports the association between BV and HPV infection, highlighting the necessity of a nine-valent vaccine.

However, it is crucial to acknowledge the limitations inherent in this study. Firstly, it was a retrospective study conducted at a single center, which may have resulted in restricted and incomplete data collection for certain factors. Consequently, the findings may solely reflect the prevalence of vaginal microbiota and HPV infection within our institution. Nevertheless, the inclusion of patients from different regions, facilitated by our hospital's three branches located in the capital of China, adds some diversity to the study population. To address these limitations, we intend to undertake a prospective randomized controlled study with a larger sample size in the future, thereby enabling a more comprehensive and robust analysis of the correlation between vaginal microbiota and HPV infection. Secondly, despite the inclusion of a sufficient number of participants, the relatively balanced prevalence rate of HPV infection may not provide enough data in each group to draw definitive clinical significance. Nonetheless, notwithstanding these limitations, we firmly believe that our study contributes to a better understanding of the correlation between vaginal microbiota and HPV infection. In conclusion, while our study has limitations, it serves as a valuable starting point and underscores the necessity for further research in this domain.

5. Conclusions

In conclusion, our findings suggest a potential association between the presence of clue cells and sialidase in the examination of vaginal microecology and HPV infection. However, it is important to note that our study design was cross-sectional, limiting our ability to establish a causal relationship between BV and HPV, despite observing a higher prevalence of HPV16 and HPV18 among participants with BV. To validate these findings, further investigations involving prospective, large-scale studies, animal models, and cell models are warranted. Moreover, considering the potential implications of both vaginal microbiota and HPV infection on CIN and cervical cancer, this research emphasizes the significance of maintaining a healthy vaginal microbiota and advocating for the use of the nine-valent HPV vaccine.

Availability of Data and Materials

The data used to support the findings of this study are available from the corresponding author upon reasonable request.

Author Contributions

XC and HL contributed equally to this work. XC, HL and JS designed the research study. XC, HL, JM, YW and JS performed the research. XC, HL and JM provided help and advice on the PCR experiments. XC and JM analyzed the data. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

All subjects gave their informed consent for inclusion before they participated in the study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of Beijing Friendship Hospital, Capital Medical University (approval number: ICDC-202113).

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Conflict of Interest

The authors declare no conflict of interest.

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