

ORIGINAL RESEARCH

The effect of vaginal dysbiosis on HPV infection and preinvasive cervical epithelial lesions

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Abstract

Background: Cervical intraepithelial lesions, and subsequently cervical cancer, are significantly caused by persistent infection with high-risk human papillomavirus (HrHPV) types. Lactobacillus-rich vaginal microbiota may protect against HPV, according to recent studies on HPV persistence and clearance. The goal of this study was to determine the effects of probiotic vaginal microbiota regulation on cervical cytology and HPV persistence in women with HPV infection and/or preinvasive cervical lesions. **Methods:** This study retrospectively examined 142 women who had positive HPV tests. 67 participants received vaginal probiotic therapy (treatment group), while 75 were managed conservatively (control group). Cervical cytology and HPV genotyping were conducted at baseline and 6th month follow-up in all patients. Probiotic strains included *Lactobacillus* (*L.*) *regenerans*, *L. helveticus*, *L. rhamnosus*, *L. salivarius*, and *Bifidobacterium* (*B.*) *longum*. **Results:** Baseline characteristics showed no significant differences between groups. In 65.7% of the treatment group and 73.3% of the control group, cytological improvement (from $\geq$ Atypical squamous cells of undetermined significance (ASC-US) to normal) was observed (for all $p > 0.05$). HPV-16 clearance was observed in 47.8% of the treatment group and 55.6% of the control group. There was little variation in HrHPV clearance rates (47.5% versus 48.3%). Only age significantly correlated with persistent HrHPV infection in multivariate analysis ($p = 0.020$). **Conclusions:** Cytological improvement and HPV clearance showed a non-significant trend with vaginal probiotic treatment. Age is a significant factor affecting the persistence of HrHPV. Longer follow-up in further randomized controlled studies is necessary to validate the potential benefits of microbiota-targeted therapies.

Keywords

Cervical cancer; Cervical cancer screening; Cervical community state type; Human papillomavirus; Pap smear; Vaginal microbiota

1. Introduction

The Human Papillomavirus (HPV) is the leading cause of cervical cancer [1]. High-risk HPV (HrHPV) is the main cause of cervical cancer and its precancerous stage, cervical intraepithelial neoplasia, and is the most common reproductive system viral infection [2]. Different grades of squamous intraepithelial lesions, at a histological level, are a consequence of persistent HrHPV infection. High-grade cervical lesions and cancer may develop from undetected, untreated HPV infection in 5–15 years [3].

The potential connection between vaginal microbiota (VM) and gynecologic cancer has been investigated in recent studies [2, 4, 5]. Variations in VM may impact the local immune response and contribute to cervical oncogenesis and HPV clearance [1, 2, 6]. Specific lactobacilli species in VM could play a protective role against opportunistic infections and be a fresh target for treatment [4, 7]. A decade of research reveals

the variable nature of VM, with recent findings indicating specific bacteria may protect against HPV and lesions [1, 8, 9]. Furthermore, pinpointing the bacterial microbiomes linked to HPV-related diseases could be clinically important, opening doors to new treatments [1, 2, 4]. High-capacity technologies now allow for rapid VM analysis, and these tools should be implemented in longitudinal studies to investigate the roles of specific bacterial species in preventing, progressing, or regressing HPV-associated cervical, vaginal, and vulvar pathologies [8, 10]. Specific bacteria could trigger illness. Understanding the influence of VM composition shifts (dysbiosis) on HPV infection and persistence may therefore lead to better infection outcome prediction [8, 10].

The goal of this study was to determine the influence of probiotic vaginal microbiota regulation on cytological results and the persistence of HPV in women with HPV infection or precancerous cervical changes.

2. Methods

2.1 Participants of the study

From January 2017 to March 2022, the study recruited participants from the gynecology outpatient clinic at Hacettepe University Faculty of Medicine Hospital. Following the Declaration of Helsinki, and with Institutional Ethics Committee approval (Decision No: 2022/08-57), all participants gave informed consent to the study.

An initial screening for the study included 270 women who had previously tested positive for HPV (Fig. 1). Exclusion criteria included pregnancy, immunosuppressive disorders, being Human Immunodeficiency Virus (HIV)-positive and incomplete clinical records. An eligibility assessment resulted in the exclusion of 43 participants for various reasons: incomplete data (17), loss to follow-up (11), pregnancy (9), or prior cervical surgery/immunosuppression (6), aligning with pre-defined criteria. After exclusions, 162 women remained eligible for inclusion. Because spontaneous clearance was much higher in women aged 20–30, the study analyzed 142 participants.

Based on their clinical management pathway, participants were split into two groups. A total of 67 women with positive HPV tests and/or abnormal cervical cytology who were receiving vaginal flora-regulating treatment made up the treatment

group. The control group consisted of 75 patients without VM treatment.

Participants underwent baseline cervical cytology and HPV genotyping and were evaluated for follow-up at 6 months. Repeat cervical smears and HPV tests provided follow-up data, facilitating longitudinal assessment of cytological regression and viral persistence or clearance.

2.2 Data collection

Demographic data (age, smoking status, parity) and contraceptive use (combined oral contraceptives pills (COC) and intrauterine device (IUD)) were collected through medical records. Following the start of vaginal flora-regulating treatment for various reasons, outpatient clinic physicians evaluated the participants and assigned them to the treatment group. Pre-probiotic vaginal capsules (containing *L. regenerans* (Vagiflora®), *L. helveticus*, *L. rhamnosus*, *L. salivarius*, and *Bifidobacterium longum* (Motiflor®)) preserved VM homeostasis in the treatment group. These products, which are quite alike, are given to patients as vaginal flora regulators during routine check-ups at the outpatient clinic. The control group consisted of 75 women who received no VM treatment.

Cervical screening and follow-up programs used the Cytobrush technique to collect Papanicolaou test (Pap smear)

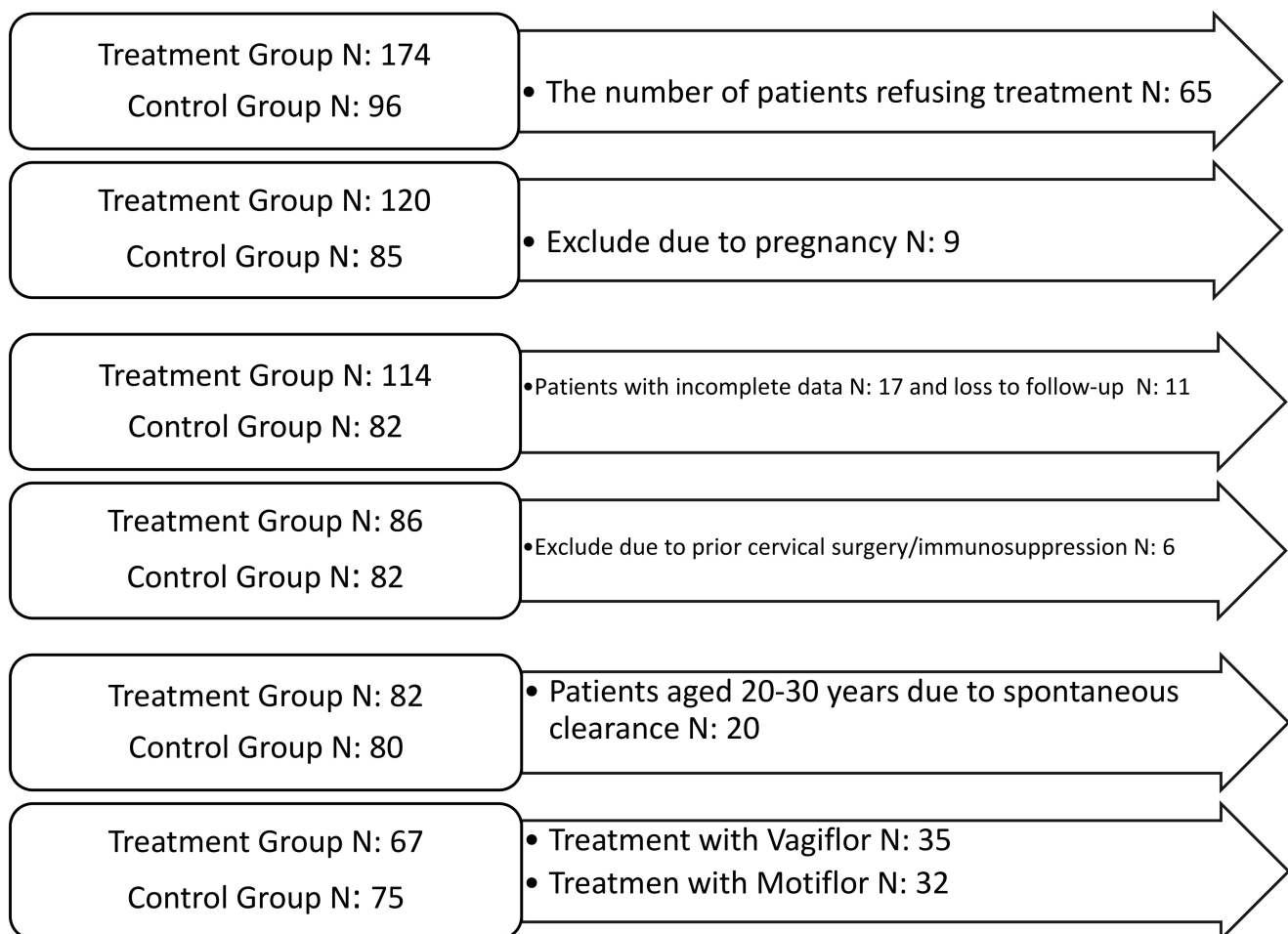


FIGURE 1. Flow chart according to study groups.

samples from patients in outpatient clinics [11]. Using the Bethesda system [11], laboratory analysis categorized the cervical Pap smear samples [12]. According to the Bethesda system, the results were categorized as Atypical squamous cells of undetermined significance (ASC-US), Low grade squamous intraepithelial lesion (LSIL) and High grade squamous intraepithelial lesion (HSIL) [12]. HrHPV testing was performed at our Microbiology laboratory using the Polymerase Chain Reaction (PCR) method. Classification of participants was determined by HPV type count (1, >1, or negative), while HPV-16 and HPV-18 changes were categorized as “converted to negative”, “persistent”, or “persistent with new HPV”. The HrHPV types detected include: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68.

2.3 Statistical analysis

Statistical significance of the data was assessed using Statistical Package for the Social Sciences program (SPSS) (version 18, IBM Corp., Armonk, NY, USA). Kolmogorov-Smirnov/Shapiro-Wilk tests determined the compatibility of continuous and descriptive variable data with a normal distribution. Frequency (n) and percentage (%) showed categorical variables; median (Interquartile range (IQR): 25th–75th percentile) represented continuous variables. Comparisons of categorical variables were performed using Pearson’s chi-square or Fisher’s exact tests; *post-hoc* Bonferroni correction was applied as needed. For dependent groups with categorical variables, the McNemar test performed repeated analysis. Multivariate logistic regression analysis determined risk factors associated with persistent HrHPV infection. Results are shown as 95% confidence intervals (CIs) and odds ratios (OR). Results with $p < 0.05$ were deemed statistically significant.

3. Results

3.1 Demographics and reproductive characteristics

The study involved a total of 142 women. An analysis of variables by age group is presented in **Supplementary Table 1**. Significant differences between age groups were pinpointed using a *Post-Hoc* Bonferroni Correction. Parity significantly associated with age ($p < 0.001$) across age groups (<30, 30–40, 40–50, ≥50 years), showing nulliparity most frequent in the under 30 group. HPV-16 clearance varied significantly across age groups ($p = 0.025$), showing the highest rate in 30–40-year-old women (69%) and the lowest in women aged 50 and above (12.5%). While a trend toward less regression in older women was seen, there were no statistically significant age-related differences in the post-treatment regression of ASC-US, LSIL, or HSIL.

Of the individuals participating in the study, 67 were in the treatment group and 75 were in the non-treatment group. Median ages were similar across groups (38 (IQR 34–46) vs. 39 (IQR 35–44) years; $p = 0.363$), and no significant differences in smoking or parity were observed (Table 1). Strikingly, IUD use was far more common among the non-treatment group (17.2%) compared to the treatment group

(1.7%), ($p = 0.010$), whereas COC use showed no significant difference ($p > 0.999$). Follow-up showed similar HPV burden distribution in both groups. Of the treatment group, 37.3% had a single HPV type, while 35.8% had multiple types, and 26.9% tested negative for HPV. A similar trend was observed in the control group (42.7%, 33.3%, and 24%, respectively) ($p = 0.806$).

TABLE 1. Comparison of demographic and reproductive characteristics between treatment and non-treatment groups.

Variables	Treatment group n = 67, n (%)	Non-treatment group n = 75, n (%)	<i>p</i> value
Age (yr)*	38 (34–46)	39 (35–44)	0.363
Smoking	51 (76.1)	53 (70.7)	0.587
Parity [‡]			
0	20 (35.7)	15 (22.1)	0.139
≥1	36 (64.3)	53 (77.9)	
COC [‡]	2 (4.0)	3 (5.3)	>0.999
IUD use [‡]	1 (1.7)	10 (17.2)	0.010[‡]
HPV count at the time of follow-up			
1	25 (37.3)	32 (42.7)	0.806
>1	24 (35.8)	25 (33.3)	
Negative	18 (26.9)	18 (24.0)	

Notes: *Numeric variables were presented as median (IQR).
[‡]Parity, COC and IUD use information of some participants could not be obtained.

[‡]: Data with significant *p* values are shown in bold.

Abbreviations: COC: Combined oral contraceptive; HPV: Human Papillomavirus; IQR: Interquartile Range; IUD: Intrauterine Device.

3.2 Cervical cytology before and after treatment

Table 2 presents the pre- and post-treatment smear results for both treatment and control groups, highlighting the distribution of ASC-US, LSIL, and HSIL regression. Among participants with initially abnormal ASC-US results, 65.7% in the treatment group and 73.3% in the control group achieved normal smears post-treatment, a non-significant difference ($p = 0.169$ and $p = 0.327$, respectively). The same trends appeared in LSIL and the categories. The treatment group showed an 80.6% LSIL normalization rate ($p > 0.999$), compared to 78.7% in controls ($p = 0.481$). Treatment and control groups showed comparable HSIL regression, with normalization rates of 97% and 97.3%, respectively ($p > 0.999$ in both).

3.3 HPV type-specific changes

Table 3 displays the analysis of HPV status modifications in patients’ post-treatment. HPV-16 analysis showed viral clearance in 47.8% of treated patients versus 55.6% in the

TABLE 2. Comparison of pre- and post-treatment cervical smear results in treatment and non-treatment groups.

Treatment group smear results (n = 67)	Smear status after treatment n (%)			
	Normal	Abnormal	Total	<i>p</i> value
Smear status before treatment				
Normal (<ASC-US)	27	9	36 (53.7)	0.169
Abnormal (≥ASC-US)	17	14	31 (46.3)	
Total	44 (65.7)	23 (34.3)	67 (100.0)	
Normal (<LSIL)	46	8	54 (80.6)	>0.999
Abnormal (≥LSIL)	9	4	13 (19.4)	
Total	55 (82.1)	12 (17.9)	67 (100.0)	
Normal (<HSIL)	62	3	65 (97.0)	>0.999
Abnormal (≥HSIL)	2	0	2 (3.0)	
Total	64 (95.5)	3 (4.5)	67 (100.0)	
Control group smear results (n = 75)	Non treatment n (%)			
	Normal	Abnormal	Total	<i>p</i> value
Smear status before treatment				
Normal (<ASC-US)	39	10	49 (65.3)	0.327
Abnormal (≥ASC-US)	16	10	26 (34.7)	
Total	55 (73.3)	20 (26.7)	75 (100.0)	
Normal (<LSIL)	52	7	59 (78.7)	0.481
Abnormal (≥LSIL)	11	5	16 (21.3)	
Total	63 (85.4)	12 (14.6)	75 (100.0)	
Normal (<HSIL)	72	1	73 (97.3)	>0.999
Abnormal (≥HSIL)	1	1	2 (2.7)	
Total	73 (97.3)	2 (2.7)	75 (100.0)	

Abbreviations: ASC-US: Atypical Squamous Cells of Undetermined Significance; HSIL: high-grade squamous intraepithelial lesion; LSIL: low-grade squamous intraepithelial lesion.

TABLE 3. Comparison of HPV type-specific changes between treatment and control group.

HPV changes	Group	Converted to negative n (%)	Persistent n (%)	Persistent with new HPV n (%)	Total n (%)	<i>p</i> value*
HPV-16 changes (n = 50)						
	Control group	15 (55.6)	10 (37.0)	2 (7.4)	27 (100.0)	0.663
	Treatment group	11 (47.8)	8 (34.8)	4 (17.4)	23 (100.0)	
HPV-18 changes (n = 9)						
	Control group	2 (100.0)	0 (0.0)	0 (0.0)	2 (100.0)	>0.999
	Treatment group	4 (57.1)	2 (28.6)	1 (14.3)	7 (100.0)	
HrHPV changes (n = 119)						
	Control group	29 (48.3)	20 (33.9)	11 (18.6)	60 (100.0)	0.995
	Treatment group	28 (47.5)	20 (33.3)	11 (18.3)	59 (100.0)	
		Converted to negative n (%)	One of HPV-16 or HPV-18 converted to negative n (%)		Total n (%)	<i>p</i> value**
HPV-16/HPV-18 changes (n = 6)						
	Control group	0 (0.0)	2 (100.0)		2 (100.0)	0.467
	Treatment group	2 (50.0)	2 (50.0)		4 (100.0)	

*Pearson Chi-square Test, Fisher's Freeman Halton Exact Test, Fisher's Exact Test.

**Pearson Chi-square Test, Fisher's Exact Test.

Abbreviations: HrHPV: High risk Human Papillomavirus; HPV: Human Papillomavirus.

control group ($p = 0.663$). HPV-18 clearance rates were 57.1% in the treated group and 100% in the untreated group ($n = 9$); however, the sample size was small. Similar negative conversion rates were observed in the treatment (47.5%) and control (48.3%) groups for HrHPV ($p = 0.995$). Moreover, the emergence of new HrHPV types was observed in 18.3% of the treatment group and 18.6% of the non-treatment group.

HPV-16/HPV-18 co-infection changes ($n = 6$) were analyzed as well. Fifty percent of treated women fully cleared the virus, compared to zero percent in the control group. However, this difference lacked statistical significance ($p = 0.467$).

3.4 Logistic regression analysis

Logistic regression determined the risk factors associated with HrHPV outcomes (Table 4). Persistent HrHPV infection was significantly predicted by age in a multivariate logistic regression model (OR: 1.057; 95% CI: 1.009–1.108; $p = 0.020$), indicating an increase in age by one unit results in a 1.057-fold increase in the probability of being HrHPV positive or persistent. Smoking, parity, COC, and IUD use showed no significant association with HrHPV persistence (Table 4).

TABLE 4. Logistic regression results for HrHPV risk factors.

Variables	Univariate analysis			
	p value	OR	95% CI	
Age	0.020[¥]	1.057	1.009	1.108
Smoking	0.583	0.796	0.352	1.798
Parity (≥ 1)	0.850	1.086	0.464	2.541
COC	0.926	1.100	0.148	8.178
IUD use	0.255	2.667	0.492	14.445

Abbreviations: CI: Confidence Interval; COC: Combined oral contraceptive pills; OR: Odds Ratio; IUD: intrauterine device.

[¥]: Data with significant p values are shown in bold.

4. Discussion

Cervical health is significantly impacted by the VM, influencing both HPV infection susceptibility and elimination. Ravel *et al.* [13] showed that specific vaginal bacterial communities, especially Lactobacillus-dominant ones, protect against sexually transmitted infections like HPV, highlighting the concept of community state types. Therefore, maintaining or reviving a healthy VM could be a good way to lower the chances of getting and worsening HPV-related cervical intraepithelial neoplasia and cervical cancer.

Practical, cost-effective ways to reduce the burden of HPV-related disease may arise from a better understanding of the VM. By studying the influence of specific probiotics on vaginal microbiota, our research contributes to the understanding of HPV and cervical cell changes. Smear results showed some improvement after treatment, most significantly in ASC-US cases, this improvement lacked statistical significance. Similarly, the treatment group experienced slightly better HPV

clearance, but this improvement was not statistically significant compared to the control.

The connection between VM composition and HPV infection is under active investigation [1, 6, 8, 14]. High levels of Lactobacillus, such as *L. crispatus* and *L. gasseri*, result in a more acidic vaginal pH, which reduces pathogen growth and improves local immune function [1, 10]. Conversely, dysbiosis results in a microbiota abundant in diverse anaerobes, triggering inflammation, compromising epithelial barriers, and promoting persistent HPV [9]. In this context, a Lactobacillus-dominant VM, either restored or maintained, could improve HPV clearance. Vitro studies show *Lactobacillus regenerans*' ability to process glycogen into lactic acid, creating a lower pH environment that could discourage the growth of pathogens and viruses [15].

Research has examined the potential of several probiotic formulations to modulate vaginal flora and influence cervical health [6, 14, 16]. Vaginal capsules of *L. regenerans* (Vagiflora®), *L. helveticus*, *L. rhamnosus*, *L. salivarius*, and *Bifidobacterium longum* (Motiflor®) were given to participants in the treatment group of our study. Promising results from similar probiotic strains are documented in the literature [6, 8, 14, 17]. In a study following bacterial vaginosis treatment, *L. casei rhamnosus* use led to VM restoration in 83% of women in the probiotic group, showing at least a 5-point Nugent score decrease [18].

There was no notable decline in ACS-US, LSIL or HSIL cases. Consistent with other studies, six months of DeFlagyn® probiotics showed an 80.9% cytological improvement rate [16], while a three-month gel treatment yielded 53% HrHPV negativity [16]. Six months after using Yakult®, 29.2% of patients were HPV-negative, with 50% showing better cytologic results than the 29.6% in the control group [19]. REBACIN® demonstrated a 74.73% HPV clearance rate in a meta-analysis [20].

In our group, the difference in clearance rates for HPV-16 (47.8% vs. 55.6%), HPV-18 (57.1% vs. 100%), and general HrHPV (47.5% vs. 48.3%) between treatment and control groups was not statistically significant. In contrast, a randomized, double-blind, placebo-controlled trial of *Lentinula edodes* mycelia-derived (Active Hexose Correlated Compound) reported a 58.8% HPV clearance rate, compared to 10.5% in the placebo group after a year [14]. The shorter follow-up and potentially insufficient treatment duration in our study might explain the lower efficacy of the probiotic therapy compared to the recent studies.

Smoking alters mucosal immunity and VM composition. Lowered functionality of neutrophils and macrophages, and reduced cytokine production due to smoking, could lead to sustained HPV infection and reduced Lactobacillus levels [21]. Our study revealed strikingly similar high smoking rates—72.5% in the treatment group and 72% in the control group. Even with this, smoking did not show a significant association with persistent HrHPV infection. The result might be explained by insufficient statistical power or the impact of other variables. Conversely, other research, like the Carboxy-Methyl-Beta-Glucan trial, showed only marginally reduced smoking (53.1% treatment, 52.2% control), but negatively affected microbes and HPV [22]. Further investigation is

warranted to clarify the effects of smoking on cervicovaginal microbiota profiles and HPV clearance in light of these conflicting results. Larger, stratified cohorts considering microbial diversity and immune markers are needed.

Our analysis revealed no significant link between parity, IUD use, or COC use, and HPV persistence, even though IUD use was more common in the untreated group. The influence of IUDs on the immune system and their potential link to cervical cancer prevention is unclear, as evidenced by inconsistent findings in the literature [23, 24].

Multivariate logistic regression revealed age as the sole significant predictor of persistent HrHPV infection (OR: 1.057; 95% CI: 1.009–1.108; $p = 0.020$). The rate of HPV-16 clearance was reduced in women over 50 (12.5%), with age correlating to a slower clearance rate, whereas the 30–40 age group had the highest clearance (69%). This data is consistent with previous research indicating that age-related immunosenescence, hormonal changes, and VM changes can hinder viral clearance [9, 25].

Although the overall trends were not statistically significant, the potential benefit of probiotic therapy may be more pronounced in specific subgroups. For example, women with ASC-US cytology or those in the younger age strata appeared to have higher rates of cytological regression and HPV clearance [1, 8, 17]. Smokers, known to have disrupted VM profiles, may also represent a target population. These findings suggest that future randomized controlled trials should consider stratification by such subgroups.

Key strengths of our study include a well-defined cohort, the use of clinically meaningful endpoints (HPV type-specific clearance and cytological regression), and consistent follow-up. Some limitations, however, need to be considered. Causal inferences are impossible given the observational design. This study was observational and non-randomized in nature. Treatment decisions were based on clinical discretion, which may introduce selection bias. Although the baseline characteristics were generally balanced, unmeasured confounders could still have influenced the observed outcomes. Second, the absence of microbiome sequencing data restricts our capacity to correlate with microbial composition and outcomes. Standardization of probiotic formulations and objective adherence measurement were lacking across participants. Furthermore, while the groups were demographically similar, uncontrolled variables, such as sexual practices, diet, and stress levels—known to impact microbiota and HPV—were not considered. Another important limitation is the lack of objective microbiome data such as Nugent scoring, vaginal pH, or 16S rRNA sequencing. This precluded direct correlations between microbiota composition and clinical outcomes. Future studies should integrate such microbiological profiling to better understand the mechanisms of probiotic action. Finally, longer-term follow-up could be necessary to assess the delayed effects of microbiota modulation on HPV outcomes. To better define probiotics' therapeutic value in managing HPV, more randomized controlled trials are needed, using larger groups and longer follow-up periods. Personalizing medicine by integrating VM profiling (such as 16S rRNA sequencing or metagenomics) would pinpoint patients most likely to respond to specific microbial therapies. Additionally, using probiotics alongside

immune-modulatory therapies or HPV vaccines could boost their effectiveness.

5. Conclusions

Despite lacking statistically significant improvements in LSIL, HSIL, or HPV clearance with probiotic treatment compared to observation, our study showed a trend toward better ASC-US cytology and modest VM balance improvement. Larger, more robust trials are necessary given the known interactions between VM, HPV infection, the immune response, and external influences such as smoking. To clarify which groups of women benefit most from VM-modulating therapies, future studies should be adequately powered and should include thorough microbial profiling.

ABBREVIATIONS

ASC-US, atypical squamous cells of undetermined significance; CST, cervicovaginal microbiota; HPV, Human Papilloma Virus; HSIL, high-grade intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion; VM, vaginal microbiota; HrHPV, high-risk human papillomavirus; *B. longum*, *Bifidobacterium longum*; COC, combined oral contraceptives; IUD, intrauterine device; IQR, Interquartile range; CIs, confidence intervals; OR, odds ratios; *L. regenerans*, *Lactobacillus regenerans*.

AVAILABILITY OF DATA AND MATERIALS

The data underpinning the findings of this study can be obtained from the corresponding author, EK, upon a reasonable request.

AUTHOR CONTRIBUTIONS

EK—study design, writing-original draft, data curation, formal analysis, visualization, investigation, conceptualization, methodology. UA—data curation, writing-review & editing. Both authors critically revised the manuscript, agreed to be fully accountable for ensuring the integrity and accuracy of the work, and read and approved the final manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This thesis study was examined by the Hacettepe University Non-Interventional Clinical Research Ethics Committee considering the rationale, purpose, approach and method, and was approved as appropriate in terms of medical ethics (Decision No. 2022/08-57). All participants gave informed consent to the study.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

SUPPLEMENTARY MATERIAL

Supplementary material associated with this article can be found, in the online version, at <https://oss.ejgo.net/files/article/1978338440129724416/attachment/Supplementary%20material.docx>.

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