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ORIGINAL ARTICLE

Investigating the correlation between oncogenic HPV, sexually transmitted disease, and vaginal microbiota in patients with normal Pap smear

Farah Farzaneh[1], Khatereh Hekmatnia[1*], Maryam Sadat Hosseini[1], Maliheh Arab[1], Maryam Talayeh[1], Mahshid Vasef[1], Samaneh Yavari[2], Hossein Keyvani[3]

[1]Department of Obstetrics and Gynecology, Preventive Gynecology Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[2]Department of Obstetrics and Gynecology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran
[3]Department of Virology, Faculty of Medicine, Iran University of Medical Sciences, Tehran, Iran

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ABSTRACT

Background and Objectives: This study investigated the correlation between high-risk (HR) human papillomavirus (HPV), sexually transmitted disease (STD), and vaginal microbiota in patients with a normal Pap smear.
Materials and Methods: For women who were referred for their routine cervical cancer screening, in addition to co-testing, some samples were taken from the vaginal and cervical environment to check the presence of the most common STD pathogens. The diagnosis of the organisms was done by means of PCR and microbial cultures.
Results: HR HPV was detected in 67 women, and STD was positive in 80% of them, while in HR HPV negative women, this was 67%. The HPV positive group reported a significantly higher rate of STD history (92% vs. 82%) and frequency of intercourse weekly (86% vs. 3.96%) ($p<0.05$). Lactobacilli, streptococcus, and staphylococcus concentrations were significantly lower in the HPV positive group compared to the HPV negative group ($p<0.007$; OR = 4.17). *Ureaplasma urealyticum* and *Ureaplasma parvum* were significantly ($p<0.001$) more prevalent in the HPV positive group compared to the HPV negative group.
Conclusion: This study showed that the existence of other STDs and the composition of the vaginal and cervical microbiome play an important role in either the clearance or the progression of high-risk HPV.

Keywords: Human papillomavirus DNA tests; Sexually transmitted diseases; Bacterial; Microbiota; Uterine cervical neoplasms; Cellular microenvironment

INTRODUCTION

The intricate relationship between high-risk (HR) human papillomavirus (HPV), sexually transmitted diseases (STDs), and the vaginal microbiome significantly influences cervical health outcomes. HR-HPV infection is the etiological agent of most cervical cancers (1), however, its progression is not solely determined by viral presence. The vaginal microenvironment and co-infections with other STDs play critical roles in modulating the risk of viral persistence and subsequent development of cervical lesions. The normal vaginal microbiome, dominated by *Lactobacillus* species, maintains vaginal homeostasis through the generation of lactic acid and other antimicrobial agents, thereby suppressing pathogenic species (2).

*Corresponding author: Khatereh Hekmatnia, MD, Department of Obstetrics and Gynecology, Preventive Gynecology Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran. Tel: +98-2173432252 Fax: +98-2173437755 Email: khaterehhekmatnia6348@gmail.com

However, HR-HPV infection is often associated with alterations in this delicate balance, leading to dysbiosis by decreasing the abundance of *Lactobacillus* species and increasing other bacteria (3-5).

This dysbiotic state can cause HR-HPV persistence and disease progression. Specifically, studies have shown that bacterial vaginosis (BV) is considered a shift in the vaginal microbiota with a dominance of anaerobic organisms such as *Gardnerella vaginalis* and *Prevotella* spp., which is related to higher HR-HPV infection rates and consequently higher risk of cervical lesion progression (6, 7). Furthermore, the presence of BV and other vaginal infections can further disrupt the microecological balance, potentially increasing the risk of HR-HPV persistence (8).

The co-occurrence of other STDs, such as *Chlamydia trachomatis, Neisseria gonorrhoeae,* and *Mycoplasma* species, further complicates this interaction (9). These co-infections can induce inflammation and alter the vaginal microenvironment, creating conditions that favor HR-HPV persistence and progression to cervical cancer (10, 11). Current research also highlights the importance of considering ethnic and demographic variations in the prevalence and impact of HR-HPV and associated microecological changes, as these factors can influence both the vaginal microbiome and the woman's susceptibility to infection (12). Moreover, lifestyle elements such as smoking, diet, and high-risk sexual behavior can also affect the risk of HR-HPV infection and its interaction with the vaginal microbiome (13). However, the effect of vaginal microbial pathogens and microbiome on HPV infection and transmission has not yet been fully confirmed (14). Lactobacilli are major components of the vaginal microflora, which along with the production of lactic acid, bacteriocins, and biosurfactant agents protect the host from pathogenic bacteria (15, 16).

Hence, this study investigated the correlation between high-risk HPVs, STDs, and the defensive role of the vaginal and cervical microbiomes in the persistence of high-risk HPV.

MATERIALS AND METHODS

This study was conducted on women aged 27 to 49 years who were referred for their routine cervical cancer screening to the gynecology clinics of Imam-Hossein Hospital from October 2023 to March 2024. Inclusion criteria: no history of genital neoplasia, cervical surgeries, loop electrosurgical excision procedures (LEEP), or cold knife conization; abnormal Pap smear or colposcopy, or abnormal uterine bleeding; nonuse of oral or topical antibiotics, ointments, probiotics, and vaginal suppositories 72 hours before sampling; no sexual intercourse in the past 48 hours; not pregnant; and no sufficient well-prepared samples, and the patient was unwilling to participate.

After describing the aim of the study, written informed consent was taken from all patients (ethical code: IR.SBMU.RETECH.REC.43011403). Willing participants and eligible participants completed questionnaires detailing demographics, socioeconomic status, medical, and sexual history. Then, for each participant, in addition to co-testing (thin prep technique Pap smear and HPV Real-time polymerase chain reaction (PCR) test), additional samples were taken from the vaginal and cervical environment for checking the presence of the most common STD pathogens, including *Chlamydia, Mycoplasma genitalum, E. coli, Klebsiella granulomatis, Ureaplasma urealyticum, Ureaplasma parvum, Neisseria gonorrhoeae, Trichomoniasis vaginalis,* Herpes Simplex Virus (HSV)-1/2, and *Candida*. All samples were collected by one gynecology oncology fellowship and taken to the laboratory.

The definitive and final diagnosis of the organisms was done by means of PCR. In addition to the PCR, standard microbial cultures were used to isolate the microorganisms. The methods are briefly mentioned here. The first step was taking several samples from the vaginal environment using Dacron swabs. Then, the swabs were immediately transferred with Stuart charcoal medium to the hospital laboratory: one swab was used for Gram staining and microscopic examination. The other four swabs were placed in Stuart charcoal medium, Thayer–Martin medium, pleuropneumonia-like organisms (PPLO) media, and chocolate agar culture. If further diagnostic test was needed, Sabouraud Dextrose Agar (SDA) with chloramphenicol culture was applied.

After excluding women with abnormal cytology, 139 women remained; their samples were assessed regarding HPV, STD pathogens, and microbiome. According to the result of the HPV test, the women were categorized into two groups:

1- HPV positive (at least one HR subtype of HPV, including 16, 18, 39, 35, 33, 31, 68, 66, 51, 52, 63, 45, 56, 58, or 59 was positive)

2- HPV negative (not positive for HR subtypes of HPV)

According to the results of STDs, the women were categorized into two other groups:

1- STD positive

2- STD negative

The basic patient information was recorded in a checklist. This included: age, age of first intercourse, and nationality (Iranian or Afghan); (2) Socioeconomic Variables: age at marriage (years), education level (primary, secondary, or higher), and residential location (urban or rural); (3) Medical History: underlying diseases (diabetes, hypertension, autoimmune disorders, and other specified conditions), history of infections (STDs at least 2 times a year requiring treatment, UTIs, and other specified infections), parity (number of live births), smoking status (current, former, or non-smoker), and drug use (prescription or illicit, specifying drug types if recorded); and (4) Sexual History: contraceptive method used (including OCP or condom or another method of contraception), number of lifetime sexual partners, BMI, parity, marital status, frequency of sexual intercourse (≥ 2 times a week), number of sexual partners, use of OCP and emergency pills in the past year, type of delivery (C/S or NVD), history of ectopic pregnancy or abortion, perineal hygiene, and use of hygiene gel or detergent. The study outcomes involved the prevalence of pathogens and microbiomes in the two HPV groups (positive and negative), and it determined the relationship between STDs and the persistence of HR HPV.

Python 3.13.0 software was used to analyze the data using descriptive statistics. The relationship between risk factors, pathogens, microbiomes, and HPV was examined using the Chi-square test and Fisher's exact test, and the independent Student's t-test was used to evaluate two quantitative variable groups.

RESULTS

Of the 139 eligible patients with normal cytology, 67 tested positive for high-risk HPV (80% of whom also tested positive for STD) and 72 tested negative for high-risk HPV (68% of whom also tested positive for STD).

The education level, place of residence, race, smoking, BMI, underlying diseases, parity, method of delivery, method of contraception, and the use of hygiene agents were similar between the two groups. Miscarriage, infertility rates, and nulliparity frequency were more prevalent in women with positive HPV results compared to those with negative results, but no significant difference was found among the groups. Similarly, there were no significant variations in rates of natural vaginal delivery versus cesarean section (Table 1).

In HPV positive group, the mean age was 29.66 ± 3.13 years, and the mean age of first intercourse was 21.19 ± 3.79 years, while in HPV negative group, the mean age was 33.46 ± 5.02 years, and the mean age of first intercourse was 25.10 ± 4.75 with a significant difference ($p<0.001$) among the two study groups indicating a higher prevalence of HPV and STDs at younger ages and in those who had had their first sexual intercourse at younger ages (Table 1).

The frequency of OCP and emergency contraceptive pill use at least once in the past year was 93.75% in the HPV positive group compared to 30% in the HPV negative, with significant differences (p=0007, OR=0.13; CI=0.05-0.32). The positive history of STDs was 92% in the HPV positive group compared to 82% in the HPV negative, with significant differences (p=0.009, OR=0.1, CI=0.07-0.4). In the HPV/STDs positive group compared to the HPV/negative STDs positive, the average of frequent sexual intercourse was 96.3% compared to 86%, which indicates a significant difference (p=0.005). However, in both study groups, a reduction in the concentration of the microbiome and normal flora was detected. A significant difference was detected in the concentration of lactobacilli in the HPV positive group (3.9% vs 64%, p=0.007, OR=4.17). *Ureaplasma urealyticum* and *Ureaplasma parvum* were significantly more prevalent in the +/HPV+ group compared to the +/HPV- group (24.07% vs. 10% and 37.03% vs. 12%, respectively; $p<0.001$). Conversely, *Candida* and *Trichomonas* strains were more common in the HPV- group (Table 2).

DISCUSSION

Lactobacilli, streptococcus, and staphylococcus concentrations were significantly lower in the women with HR HPV, While *Ureaplasma urealyticum* and *Ureaplasma parvum* concentrations were significantly higher in these women compared to HPV negative women. These differences in vaginal microbiome and microbial pathogens result in non-clearing HR HPV. In line with our findings, Eser et al.'s

Table 1. Comparison of baseline characteristics of the patients with positive or negative HPV

Variable	HPV Positive	HPV Negative	P-Value	ODDS Ratio	CI 95% (Lower – Upper)
Age	29.66 ± 3.13	33.46 ± 5.02	<0.001	-	-
Age at first intercourse	21.19 ± 3.79	25.10 ± 4.75	<0.001	-	-
Residency					
Tehran	15 (27.78%)	15 (30.0%)	1.000	1.000	0.210 - 4.758
Afghanistan	4 (7.41%)	4 (8.0%)			
Near Tehran	35 (64.81%)	31 (62.0%)			
Highly educated	37 (68.52%)	24 (48.0%)	0.054	0.424	0.191 - 0.942
Smoker	25 (46.3%)	15 (30.0%)	0.132	0.497	0.222 - 1.115
OCP user	41 (75.93%)	15 (30.0%)	<0.001	0.136	0.057 - 0.324
Multi-partner	37 (68.0%)	26 (52.0%)	0.028	0.382	0.172 - 0.847
Comorbidity positive	23 (42.59%)	17 (34.0%)	0.362	0.634	0.284 - 1.415
Parity			0.099	2.297	0.877 - 6.013
Nulliparity	21 (38.89%)	16 (32.0%)			
Multiparty	12 (22.22%)	21 (42.0%)			
Abortion	9 (16.67%)	3 (6.0%)			
Infertility	8 (14.81%)	6 (12.0%)			
Ectopic pregnancy	4 (7.41%)	4 (8.0%)			
Vaginal gel user	31 (57.4%)	17 (34%)	0.128	2.009	0.904 - 4.465
>2 intercourse per week	52 (96.3%)	47 (94.0%)	0.002	0.243	0.103 - 0.573
History of STD	50 (92.59%)	41 (82.0%)	<0.001	0.180	0.078 - 0.419
Method contraceptive	25 (46.2%)	15 (30%)	0.140	0.48	0.222-1.114
Except (OCP)	29 (53.7%)	35 (70%)			
Method of delivery					
NVD	37 (60%)	26 (52%)	0.057	0.42	0.191-0.942
C/S	17 (31.5%)	24 (48%)			

Table 2. Comparison of STI pathogens and mycobacterium of patients with positive or negative HPV

Pathogens	HPV positive	HPV negative	P-value	Odds Ratio	CI 95 (Lower – Upper)
Lactobacillus	5 (9.26)	32 (64.0)	<0.001	17.422	5.879 - 51.631
Streptococcus	15 (27.78)	24 (48.0)	0.054	2.400	1.064 - 5.416
Staphylococcus	16 (29.63)	25 (50.0)	0.054	2.375	1.063 - 5.314
Ureaplasma urealyticum	13 (24.07)	6 (12.0)	0.014	0.002	NaN - NaN
Klebsiella	2 (3.7)	3 (6.0)	0.604	2.261	0.066 - 12.922
E. coli	4 (7.41)	9 (18.0)	0.181	0.430	0.065 - 1.237
Ureaplasma parvum	20 (37.03)	5 (10)	<0.001	0	0.064 - 0.554
Neisseria gonorrhoeae	1 (1.85)	1 (2.0)	0.461	0	NaN - NaN
Trichomonas vaginalis	4 (7.40)	10 (20)	0.020	0.583	3 (6.0)
Chlamydia trachomatis	2 (3.7)	3 (6.0)	0.930	1.660	0.266 - 10.368
Candida	4 (7.41)	9 (18.0)	0.930	1.660	0.266 - 10.368
HSV2	0	1 (2.0)	0.182	2.744	0.788 - 9.559
HSV1	1 (1.85)	0	0.969	0	NaN - NaN
Mycoplasma	2 (3.7)	3 (6.0)	1.000	1.082	0.066 - 17.768

study showed that the prevalence of STDs in women with HR HPV-positive (63.9%) was significantly higher. *Ureaplasma parvum* was the most prevalent STD (40.0%), followed by *Ureaplasma urealyticum* (28.5%), *Mycoplasma hominis* (17.2%), and *Chlamydia trachomatis* (11.4%) (17). In a study by Dou et al., it was found that the decreased prevalence of *Lactobacillus* and *Gardnerella vaginalis* was closely related to HPV infection in women (18). These results are in line with our findings.

Tantengco et al.'s study stated that HPV-16 is the most common HPV genotype in women with cervical cancer, and was associated with *Ureaplasma* co-infection in 22.3% of cases and *Mycoplasma* co-infection in 9.1% of cases (19), which are in line with our study except for some points. Perhaps the most important reasons for these differences are the differences in the type of patient selection, sampling method, and differences in sample size.

Kops et al. in their study showed that the prevalence of HPV, especially high-risk HPVs, was significantly higher in patients with at least one STD (20). HPV infection is one of the most important risk factors for cervical carcinoma. Co-infection of different HPV genotypes, such as HPV 51, 52, 16, or 33, is a public health concern for controlling this infection in the community.

It has also been shown that co-infection with HPV with STDs can increase the risk of cervical carcinoma by about 10-20% (21). Since the HPV genotyping was not performed in our study, it is not possible to fully compare it with previous studies, but the observed co-occurrence of HPV and STDs was similar to previous studies.

In a study by Paesi et al., the association of positive HPV with *Gardnerella vaginalis, Trichomonas vaginalis,* and *Candida* in the female genital tract was investigated. Although no such association was found, the study detected infection with up to five types of HPV (22). HPV is the most prevalent STD; recently, several methods have been used to control and treat it. One of the most recent methods is the use of intravaginal probiotics. However, conflicting studies have shown that the use of vaginal probiotic capsules has no impact on HPV clearance (23).

In the study by Eshtad et al., it was stated that about 9% of the total body microbiota is made up of the genital microbiota. *Lactobacillus* is the dominant microbiota of the genital tract, which creates an acidic environment that prevents the growth of harmful bacteria. The composition of the microbiota can change under the influence of factors such as age and hormones. A complete understanding of the mechanism of the genital microbiota is effective in the development, treatment, and prevention of gynecological diseases such as HPV (24). Gao et al.'s study showed higher concentrations of *Lactobacillus gasseri* and *Gardnerella vaginalis* in women with positive HPV results (25).

In addition to identifying and investigating factors that reduce lactobacilli function, such as STDs, effective interventions are needed. STDs are a common cause of microbiome reduction, leading to the disruption of the mucosal surface and repeated regeneration of the vaginal epithelium. This process reduces the host cell's flexibility and impairs the homeostatic repair process. Furthermore, this damage and inflammation, can extend to the vaginal epithelium leading to inflammation and dysplastic changes in the cervix. These cervical changes were found to be more common in the HPV positive group.

This study's limitations included the small sample size, the setting in a tertiary referral center, the cross-sectional design, and specific design limitations like the temporality effect and the inability to distinguish between prevalent and incident cases. Additionally, we only enrolled women with normal Pap smear results and did not repeat sampling in the study cases. Future research should include prospective, longitudinal, and multicenter studies involving women with different Pap smear results.

CONCLUSION

This study showed that the existence of other STDs and the composition of the vaginal and cervical microbiome play important roles in either the clearance or the progression of HR HPV. In order to prevent unnecessary and expensive colposcopy or HPV tests, current study's results suggest that gynecologists should pay attention to co-infection with other STDs in women with persistent HR-HPV.

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