Bacterial Vaginosis Decreases the Risk of Cervical Cytological Abnormalities

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ABSTRACT

Genital tract infections, including vulvovaginal candidiasis and bacterial vaginosis, have emerged as potential modulators of persistent human papillomavirus (HPV) infections causing cervical cytologic abnormalities and cervical cancer. This study aimed to investigate whether vulvovaginal candidiasis or bacterial vaginosis had an additional effect on HPV infection and thus caused such abnormalities. ThinPrep cytologic tests were used to detect cytologic abnormalities, vulvovaginal candidiasis, and bacterial vaginosis in 14,679 women. Cytologic abnormalities included atypical squamous cells of undetermined significance, low-grade squamous intraepithelial lesions, high-grade squamous intraepithelial lesions, atypical squamous cells-cannot exclude HSIL, and squamous cell carcinoma. Logistic regression Model 1 (univariate regression) and Model 2 (multivariate logistic regression analysis adjusted for age combined with HPV infection) were used to analyze the association between bacterial vaginosis and cytologic abnormalities, or vulvovaginal candidiasis and cytologic abnormalities, alone or in the presence of HPV infection. Bacterial vaginosis infection rates were found to be significantly higher in the cytology-negative group among all participants and those with HPV infection ($P = 0.003$, $P < 0.001$, respectively). Analyses using Model 1 and Model 2 both pointed to bacterial vaginosis as a protective factor against cytologic abnormalities for all participants (OR = 0.36, 0.17, respectively, $P < 0.05$) and for HPV-infected participants (OR = 0.17, 0.16, respectively, $P < 0.05$). Neither vulvovaginal candidiasis nor vulvovaginal candidiasis + HPV was significantly associated with the incidence of cytologic abnormalities based on Model 1 (OR = 0.94, 0.71, respectively, $P > 0.05$) and Model 2 (OR = 0.91, 0.74, respectively, $P > 0.05$). Furthermore, neither vulvovaginal candidiasis nor bacterial vaginosis increased the incidence of cytologic abnormalities regardless of HPV infection status, while bacterial vaginosis might possibly prevent cytologic abnormalities in women coinfected by HPV.

Prevention Relevance : Neither vulvovaginal candidiasis nor bacterial vaginosis was found to increase the incidence of cervical cytologic abnormalities with or without the presence of HPV. On the contrary, bacterial vaginosis may play a role in preventing cytologic abnormalities in women with HPV coinfection.

Introduction

Human papillomavirus (HPV) is the main cause of cervical cancer and hence is a cancer precursor (cervical intraepithelial neoplasia 3, CIN 3). HPV is also associated with CIN 1 and CIN 2, but not necessarily in a precancerous manner (1, 2). Some studies have found that only a few cases of persistent HPV infection actually progress to full blown cervical cancer and that most cases of transient HPV infections are normal or result in only mild cytologic abnormalities (1). Recently, other cofactors have emerged as potential modulators of those persistent HPV infections that progress to high-grade lesions of the cervix uteri such as cervical inflammation and other genital tract infections (GTI), including vulvovaginal candidiasis, bacterial vaginosis, *Trichomonas vaginalis*, *Chlamydia trachomatis*, and *Ureaplasma urealyticum* (3). Although GTIs have been proposed as etiologic cofactors for cervical cancer, the correlation between each single agent and cytologic abnormalities has been inconsistent in previous research (4–6). The associations between vulvovaginal candidiasis/bacterial vaginosis and cytologic abnormalities in addition to HPV infection have yet to be investigated.

It has been established that most cervicovaginal microenvironments (CVM) consist predominantly of lactobacilli and that CVM alterations can cause symptomatic conditions (7). The main cause of vaginal dysbacteriosis among those below reproductive age is bacterial vaginosis, which has been traditionally characterized by a reduction in vaginal lactobacilli and an overgrowth of other (facultative) anaerobic bacteria. Another common type of microbiological vaginitis is vulvovaginal candidiasis (8). GTIs such as vulvovaginal candidiasis and

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bacterial vaginosis appear to be directly related to the physiologic, inflammatory, and immunologic state of the cervix, thus influencing the cervical microenvironment, which has been reported to play a crucial role in the clearance of high-risk HPV (5, 9). In addition to HPV, vulvovaginal candidiasis and bacterial vaginosis are two other common pathogens that are associated with cervical cytologic abnormalities. These agents are cofactors in the pathogenesis of cytologic abnormalities and HPV transmissibility, persistence, and progression, as well as HPV-induced carcinogenesis. Epidemiologic evidence has suggested that vulvovaginal candidiasis or bacterial vaginosis coinfection with HPV may play a central role in the etiology of CIN and cervical cancer (10). However, other studies have suggested that vulvovaginal candidiasis or bacterial vaginosis infections are independent factors and that there is no association between these infections and HPV with cytologic abnormalities (11). Thus, the roles of vulvovaginal candidiasis + HPV and bacterial vaginosis + HPV as risk factors for the development of cervical lesions remain controversial.

In this cross-sectional study, participants were recruited to undergo ThinPrep cytologic tests and HPV DNA tests. Cytology allowed for the observation of both vulvovaginal candidiasis and bacterial vaginosis. A logistic regression model was used to analyze whether bacterial vaginosis or vulvovaginal candidiasis had an additional effect on HPV infection and thus caused cytologic abnormalities. The correlations between vulvovaginal candidiasis + HPV infection, bacterial vaginosis + HPV infection, and cytologic abnormalities were also explored.

Materials and Methods

Women between the ages of 18 and 80 with a history of sexual activity who visited the obstetrics-gynecology clinics of Sun Yat-sen Memorial Hospital, Sun Yat-sen University between January 2018 and May 2020 were recruited as study participants (**Fig. 1**). The characteristics of the participants are shown in **Table 1**. Women with a history of hysterectomy or who declined to participate in the study at any time for any reason were excluded. The sample size was calculated according to the formula for population rate estimation (Supplementary Materials and Methods).

In this study, cytology positive was defined according to the Bethesda System (TBS) 2004 and included test results such as atypical squamous cells of undetermined significance (ASC-US), low-grade squamous intraepithelial lesions (LSIL), high-grade squamous intraepithelial lesions (HSIL), atypical squamous cells-cannot exclude HSIL (ASC-H), and squamous cell carcinoma (SCC), which were also considered as cytologic abnormalities groups. The criterion for inclusion in the cytology-negative group was a negative for intraepithelial lesions or malignancy (NILM) test result. Patients with abnormal results were informed by trained staff via telephone and were advised to undergo further treatment at the hospital. A file was generated for each patient and an information management platform was built to screen high-risk patients.

Cytology testing

Cervical specimens were strictly sampled during periods of nonmenstruation. The participants had no operations performed within three days prior to sampling. For sampling, a cotton swab was wiped inside the cervix to obtain a secretion specimen, and a cervical brush (ThinPrep 2000; Hologic Inc.) was inserted into the external os of the cervix and rotated clockwise for 8–10 complete revolutions. The cervical brush head was then placed into a tube containing cell preservation liquid (PreservCyt Solution; Hologic, Inc.). The tubes were kept upright. Cervical liquid-based cytology

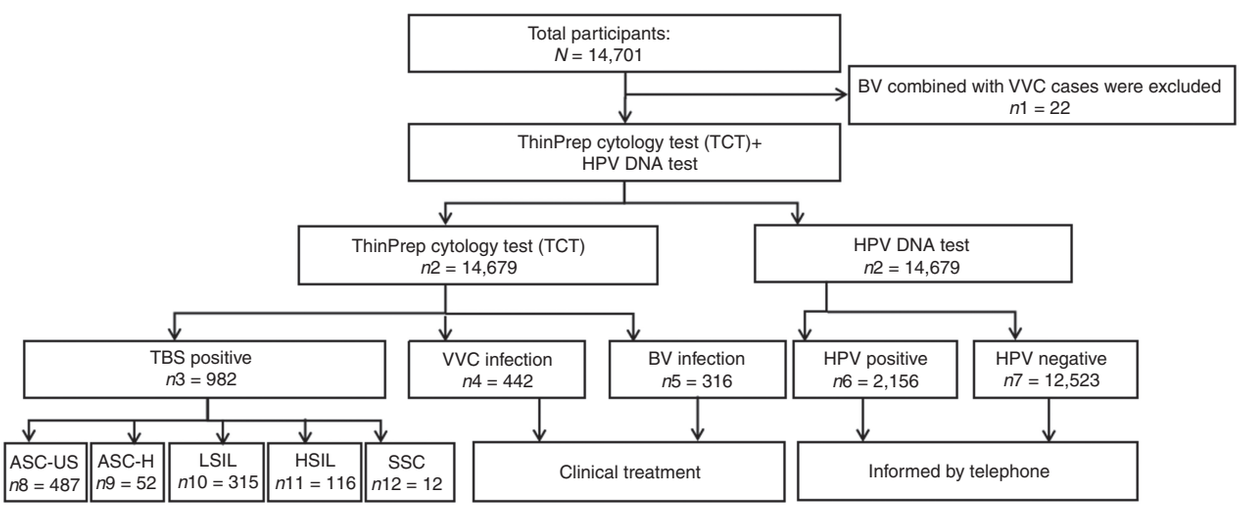

Figure 1.
Flow chart of patient recruitment. This flow chart provides statistics regarding study enrollment, allocation, follow-up methods, and analysis of cytology results, HPV, vulvovaginal candidiasis, and bacterial vaginosis infection.

Table 1. Characteristics of the study participants.

Participant characteristics	Cytology positive *N* (%)	Cytology negative *N* (%)	χ^2	*P*
Smoking status			1.175	0.278
Never	945 (96.2)	13,267 (96.9)		
Current	37 (3.8)	430 (3.1)		
Age at enrollment (years)			74.185	<0.001[a]
<50	747 (76.1)	11,794 (86.1)		
≥50	235 (23.9)	1,903 (13.9)		
Education			3.882	0.274
Primary school	133 (13.5)	1,646 (12)		
Middle/high school	412 (42)	5,781 (42.2)		
Undergraduate	393 (40)	5,502 (40.2)		
Postgraduate	44 (4.5)	768 (5.6)		
Age at sexual debut (years)			1.504	0.22
≤20	234 (23.8)	3,033 (22.1)		
>20	748 (76.2)	10,664 (77.9)		
Lifetime no. of sexual partners			504.94	<0.001[a]
1–2	470 (47.9)	10,834 (79.1)		
≥3	512 (51.1)	2,863 (20.9)		
Contraceptive methods			572.81	<0.001[a]
Condom	310 (31.6)	8,478 (61.9)		
Contraceptive pills	37 (3.8)	51 (0.4)		
IUDs (total)	187 (19)	889 (6.5)	18.08	<0.001[a]
LNG-IUS (progestin)	54 (28.9)	158 (17.8)		
Copper	106 (56.7)	643 (72.3)		
Others	27 (14.4)	88 (9.9)		
Others	448 (45.6)	4,279 (31.2)		
Monthly income (yuan)			0.832	0.362
≤5,000	741 (75.5)	10,155 (74.1)		
>5,000	241 (24.5)	3,542 (25.9)		

Note: Values are numbers (percentages) unless otherwise stated.
Abbreviation: LNG-IUS, levonorgestrel-releasing intrauterine system.
[a]$P < 0.001$.

tests were performed by experienced cytology experts at the Department of Cellular and Molecular Diagnostics Center of Sun Yat-sen Memorial Hospital. The collected exfoliated cells of all participants were stored in liquid preservative to prepare ThinPrep slides, which were prepared following standard procedures and used for HPV tests. Using the same cytology smears, bacterial vaginosis and vulvovaginal candidiasis were detected according to the diagnostic criteria of TBS 2004 (Supplementary Fig. S1).

The criteria for a shift in flora suggestive of bacterial vaginosis were as follows:

(i) Individual squamous cells were covered by a layer of coccobacilli that obscured the cell membrane, forming clue cells;
(ii) There were large numbers of inflammatory cells; and
(iii) There was a conspicuous absence of lactobacilli (Supplementary Fig. S1A).

The diagnostic criteria for vulvovaginal candidiasis included:

(i) The presence of budding yeast (3–7μm) and/or pseudohyphae; and
(ii) The pseudohyphae present were quite long, spanning many cells, and eosinophilic to gray-brown on Pap staining. Pseudohyphae, formed by the extension of blastomycete cytoplasm, lacked a true transverse septum but were constricted along the mediastinum, showing the formation of new cells. Leukocyte nuclear debris could often be seen, as well as pseudohyphae "strung together" in the rouleaux squamous epithelium. To report the conditions of the fungi (Candida), Candida pseudohyphae or blastospores were clearly observed under a microscope (other fungi were not reported; Supplementary Fig. S1B).

The presence of koilocytes, which are characterized by a wide, well-defined perinuclear lucent area (Supplementary Fig. S1C), indicated possible HPV infection. Furthermore, the absence or presence of HPV infection was definitively confirmed through the use of HPV DNA tests.

Additional ectocervical and endocervical cells were collected from participants using Dacron swabs and stored in specimen transport medium (Digene) for HPV testing.

Cytology smears with bacterial vaginosis detected were included in the bacterial vaginosis group; cytology smears with Candida detected were included in the vulvovaginal candidiasis group; and cytology smears with bacterial vaginosis and vulvovaginal candidiasis absent were included in the vulvovaginal candidiasis/bacterial vaginosis–absent group (**Tables 2** and **3**). To exclude the effect of bacterial vaginosis combined with vulvovaginal candidiasis on cytologic abnormalities, cases of bacterial vaginosis and vulvovaginal candidiasis coinfection were excluded from this study.

HPV detection method

The specimens were extracted from the liquid preservative, and HPV DNA was detected using a kit that fully automated the sample preparation and real-time PCR amplification of 25 HPV L1 genes (HPV-16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 26, 53, 66, 73, 82, 6, 11, 42, 43, 44, 81, and 83).

Table 2. Comparison of bacterial vaginosis and vulvovaginal candidiasis infection rates between cytology negative and positive groups among total participants and HPV-infected participants.

Total participants	TCT− (*N* = 13,697)	TCT+ (*N* = 982)	χ^2	*P*
BV+	308 (2.25%)	8 (0.81%)	8.945	0.003[a]
VCC+	414 (3.02%)	28 (2.85%)	0.092	0.762
HPV+	TCT− (*n* = 1,336)	TCT+ (*n* = 820)		
BV+	57 (4.27%)	6 (0.73%)	22.381	<0.001[b]
VCC+	48 (3.59%)	21 (2.56%)	1.746	0.186

Abbreviations: BV+, bacterial vaginosis infection; VCC+, vulvovaginal candidiasis infection; HPV+, HPV-infected participants; TCT−, cytology negative (NILM); TCT+, cytology positive (ASC-US, ASC-H, LSIL, HSIL, SCC).
[a]$P < 0.01$.
[b]$P < 0.001$.

Table 3. The association between bacterial vaginosis and the severity of cytologic abnormalities among total participants and HPV-infected participants.

Total participants (*n* = 14,679)	**NILM**	**ASC-US**	**ASC-H**	**LSIL**	**HSIL**	**SCC**	**Z**	***P***
BV+ (*n* = 316)	308 (97.5%)	5 (1.58%)	2 (0.63%)	0 (0.00%)	1 (0.32%)	0 (0.00%)	3.013	0.003[a]
BV− (*n* = 14,357)	13,389 (93.2%)	482 (3.36%)	50 (0.35%)	315 (2.19%)	115 (0.80%)	12 (0.08%)		
HPV+ (*n* = 2,156)								
BV+ (*n* = 63)	57 (90.5%)	3 (4.76%)	2 (3.17%)	0 (0.00%)	1 (1.59%)	0 (0.00%)	4.676	<0.001[b]
BV− (*n* = 2,093)	1,279 (61.1%)	351 (16.8%)	48 (2.29%)	293 (14.0%)	110 (5.26%)	12 (0.57%)		

Note: *P* values were calculated by a non-parametric rank sum test.
Abbreviations: BV+, bacterial vaginosis infection; BV−, bacterial vaginosis absent; HPV+, HPV-infected participants.
[a]$P < 0.01$.
[b]$P < 0.001$.

Hybridized HPV DNA was analyzed using Applied Biosystems 3500 Dx/3500xL Dx (Applied Biosystems by Genetic Analyzers, Life Technologies). HPV positivity was confirmed in all samples using HPV DNA tests. Cases of bacterial vaginosis coinfection with HPV were included in the bacterial vaginosis + HPV group, and cases of vulvovaginal candidiasis coinfection with HPV were included in the vulvovaginal candidiasis + HPV group. Cases with HPV alone, and with neither bacterial vaginosis nor vulvovaginal candidiasis infection in the sample, were included in the HPV group.

The study was registered and approved by the Institutional Review Board of Sun Yat-sen Memorial Hospital, Sun Yat-sen University (No. SYSEC-KY-KS-2019–110). The study procedures were designed and conducted in accordance with the tenets of the Declaration of Helsinki. All women who participated in the study completed and signed a consent form before the study began. A similar explanation of the study was provided to and approved by each participant prior to an experienced gynecologist collecting a Pap smear, which was used for cytology and HPV DNA testing.

Statistical analysis

SPSS 25.0 (SPSS, Inc.) was used to perform comparative and descriptive analyses. Statistical analysis was performed using the Cochran–Armitage trend test to determine cytology positivity, HPV infection, and bacterial vaginosis and vulvovaginal candidiasis according to age distribution, the results of which are in **Fig. 2A–D**. A statistical description of the detailed data is shown in Supplementary Table S1. A Cox regression model with an age timescale was fitted for bacterial vaginosis positive rates by nonlinear correlation analysis as a restricted cubic spline (smooth curve) as shown in **Fig. 2E**. The results of χ^2 tests for comparison of participant characteristics and GTI rates are displayed in **Tables 1** and **2**. A nonparametric rank-sum test was performed to evaluate the association between bacterial vaginosis coinfection with HPV and cytologic abnormalities, as shown in **Table 3**. Logistic regression Models 1 and 2 were used to analyze the correlations between both vulvovaginal candidiasis and cytologic abnormalities, and bacterial vaginosis and cytologic abnormalities, as well as the correlations between vulvovaginal candidiasis + HPV infection or bacterial vaginosis + HPV infection and cytologic abnormalities, the results of which are displayed in **Table 4**. A univariate regression analysis for prevalence of cytologic abnormalities, HPV infection, and GTIs by age distribution is shown in Supplementary Table S2. $P < 0.05$ was considered statistically significant.

Data availability statement

Data may be made available upon request due to privacy/ethical restrictions.

Results

Participants' characteristics

Between January 2018 and May 2020, a total of 14,679 participants (36 ± 9 years) underwent cytology and HPV DNA tests. Among them, 982 participants (6.69%) were found to be cytology positive (ASC-US, ASC-H, LSIL, HSIL, and SCC). The clinical characteristics of all participants between the cytology-positive and -negative groups were then analyzed. That information included age distribution (Supplementary Table S1), smoking status, education, age at sexual debut, number of sexual partners, contraceptive methods, and socioeconomic characteristics (**Table 1**). A significant difference was found between the cytology positive and negative groups in terms of cervical cancer screening occurring before and after the age of 50. No significant differences were found between groups regarding the above-mentioned aspects, except that having multiple sexual partners and contraceptive methods appeared to be associated with a higher risk of cytologic abnormalities ($P < 0.05$).

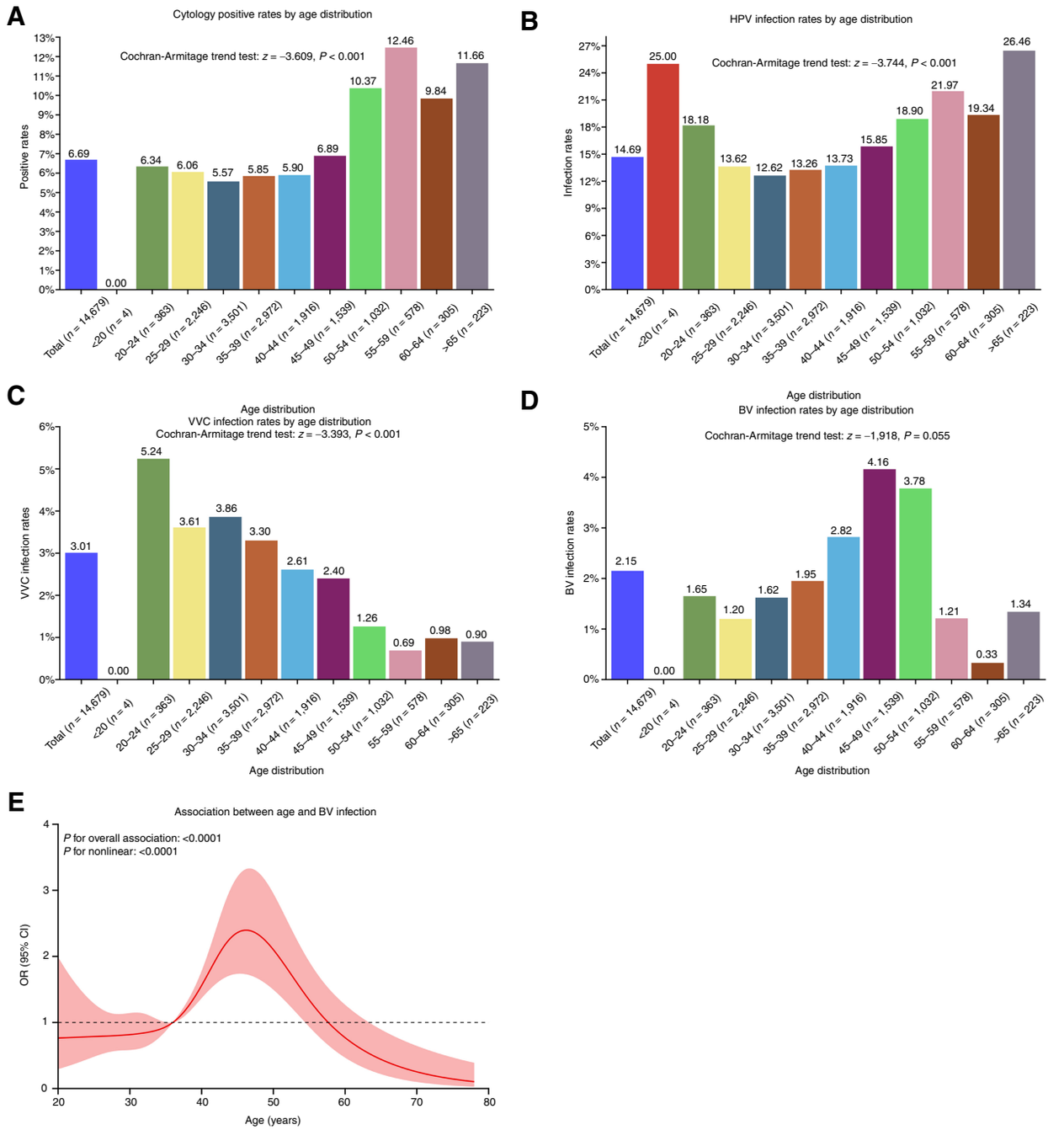

Figure 2.

Prevalence of cytologic abnormalities, HPV, vulvovaginal candidiasis, and bacterial vaginosis infection rates by age. **A,** Prevalence of cytology positive rates by age, $P < 0.05$. **B,** Prevalence of HPV infection rates by age, $P < 0.05$. **C,** Prevalence of vulvovaginal candidiasis infection rates by age, $P < 0.05$. **D,** Prevalence of bacterial vaginosis infection rates by age, $P > 0.05$. **E,** Prevalence of bacterial vaginosis infection rates by age (restricted cubic spline), $P < 0.0001$.

Prevalence of cytologic abnormalities, HPV, vulvovaginal candidiasis, and bacterial vaginosis infection rates

Among the cytology positive group, 49.59% (487/982) had ASC-US, 5.30% (52/982) had ASC-H, 32.08% (315/982) had LSIL, 11.81% (116/982) had HSIL, and 1.22% (12/982) had SCC. The overall HPV infection rate was 14.69% (2,156/14,679; Supplementary Table S1). The cytology-positive and HPV infection rates both showed a positive correlation with age distribution ($P < 0.001$; **Fig. 2A** and **B**). The rates of cytologic

Table 4. Association between candidiasis or bacterial vaginosis coinfection with HPV and cervical cytologic abnormalities.

	Model 1		Model 2	
Group	**OR (95% CI)**	***P***	**OR (95% CI)**	***P***
VVC vs. VVC absent	0.94 (0.62–1.36)	0.762	0.91 (0.57–1.41)	0.696
BV vs. BV absent	0.36 (0.16–0.67)	0.004[a]	0.17 (0.07–0.36)	<0.001[b]
VVC+ HPV vs. HPV	0.71 (0.41–1.17)	0.188	0.74 (0.43–1.22)	0.251
BV+ HPV vs. HPV	0.17 (0.06–0.36)	<0.001[b]	0.16 (0.06–0.35)	<0.001[b]

Note: Model 1, univariate regression analysis; Model 2, binary logistic regression by age + HPV infection adjusted (not adjusted in HPV population analysis, vulvovaginal candidiasis + HPV vs. HPV and bacterial vaginosis + HPV vs. HPV were only in HPV-positive people).
Abbreviations: BV, bacterial vaginosis; VVC, vulvovaginal candidiasis.
[a]$P < 0.01$.
[b]$P < 0.001$.

abnormalities and HPV infection particularly increased among women of premenopausal age (>50 and >45 years, respectively). The overall vulvovaginal candidiasis and bacterial vaginosis infection rates were 3.01% (442/14,679) and 2.15% (316/14,679), respectively (Supplementary Table S1). Vulvovaginal candidiasis positive rates were found to significantly decrease with age ($P < 0.001$, **Fig. 2C**). However, bacterial vaginosis positive rates showed no significant differences with regard to age distribution ($P > 0.05$; **Fig. 2D**). Furthermore, the rates of bacterial vaginosis infection were found to be correlated with age through a restricted cubic spline. The incidence of bacterial vaginosis was found to increase between the ages of 35 and 45 and then significantly decrease after age 45 ($P < 0.0001$; **Fig. 2E**).

In **Fig. 2**, significant trend changes can be observed in prevalence rates with age in each group. Thus, logistic regression analysis was used to compare the differences in cytology positive, HPV, vulvovaginal candidiasis, and bacterial vaginosis infection rates by age distribution. There were significant differences in all groups, as shown in Supplementary Table S2 ($P < 0.05$). The ORs in the cytology positive, HPV, and bacterial vaginosis groups were 1.023, 1.018, and 1.020, respectively. These results suggested that as age increased, the risk of cytologic abnormalities, HPV, and bacterial vaginosis infections also increased. Meanwhile, however, the risk of vulvovaginal candidiasis infection was found to be reduced with an OR of 0.961.

Prevalence of vulvovaginal candidiasis, bacterial vaginosis, and HPV infection rates among cytologic abnormalities groups

Overall, 93.67% (414/442) of participants with vulvovaginal candidiasis and 97.47% (308/316) of participants with bacterial vaginosis were cytology negative. Among all participants, bacterial vaginosis infection rates were found to be significantly higher in the cytology-negative group ($P = 0.003$), and similar results were observed among the HPV-infected participants ($P < 0.001$). The vulvovaginal candidiasis infection rates were not different between the cytology negative and positive groups in either the total participants or the HPV-infected participants ($P > 0.05$; **Table 2**).

In bacterial vaginosis infection and bacterial vaginosis absent groups, cytology results were significantly different in each cytologic abnormalities groups. About 97.5% of bacterial vaginosis infection participants had negative cytology, but 93.2% of bacterial vaginosis absent participants had negative cytology decreased slightly. Similar results were found in either total participants or HPV-infected participants ($z = 3.013$, $P = 0.003$; $z = 4.676$, $P < 0.001$, respectively). Interestingly, the proportion of bacterial vaginosis infection among all participants was higher in the low-grade cytologic abnormalities group (ASC-US, 1.58%) than in the high-grade cytologic abnormalities group (ASC-H + LSIL + HSIL + SCC, 0.95%). The proportion of participants with bacterial vaginosis infection who also had HPV infection was the same (4.76%) in both the low- and high-grade cytologic abnormalities groups. Thus, among all the participants, those with bacterial vaginosis infection showed a lower degree of cytologic abnormalities (**Table 3**).

Logistic analysis of the correlation between vulvovaginal candidiasis/bacterial vaginosis coinfection with HPV and incidence of cytologic abnormalities

To clearly understand the exact risk of vulvovaginal candidiasis or bacterial vaginosis on cytologic abnormalities, Model 1 (univariate regression analysis) and Model 2 (binary logistic regression by age + HPV infection adjusted (not adjusted in HPV population analysis)) were used to analyze the correlations between vulvovaginal candidiasis and cytologic abnormalities, bacterial vaginosis and cytologic abnormalities, and vulvovaginal candidiasis + HPV or bacterial vaginosis + HPV and cytologic abnormalities.

The data from Model 1 showed that bacterial vaginosis ($OR = 0.36$, $P = 0.004$) was a protective factor against cytologic abnormalities. In addition, data from Model 2 demonstrated a stronger association between bacterial vaginosis infection and cytologic abnormalities ($OR = 0.17$, $P < 0.001$). However, the data from Model 1 showed that vulvovaginal candidiasis ($OR = 0.94$) and vulvovaginal candidiasis + HPV ($OR = 0.71$) were not significantly associated with an increase in the incidence of cytologic abnormalities ($P > 0.05$), similarly to the data from Model 2 ($OR = 0.91$, $OR = 0.74$, $P > 0.05$). Models 1 and 2 showed that both bacterial vaginosis and bacterial vaginosis +

HPV could reduce the incidence of cytologic abnormalities ($P < 0.05$, **Table 4**).

Discussion

In this study, cytology was used to detect vulvovaginal candidiasis, bacterial vaginosis, and cytologic abnormalities, and the correlation between vulvovaginal candidiasis/bacterial vaginosis and cytologic abnormalities was subsequently analyzed. The intent was to maximize cytological screening information in order to interpret the clinical relevance of cervical lesions. In the end, neither the presence of bacterial vaginosis nor vulvovaginal candidiasis was found to be associated with an increased risk of cytologic abnormalities compared with the absence of bacterial vaginosis or vulvovaginal candidiasis. Furthermore, HPV coinfection with bacterial vaginosis or vulvovaginal candidiasis was also not found to be associated with an increased risk of cytologic abnormalities compared with HPV infection alone. Notably, bacterial vaginosis was observed to possibly have a protective role against cytologic abnormalities in women with HPV coinfection.

The relationship between the CVM and immunity based on age in patients with cervical lesions has been confirmed in several studies. With age, women are often found to have characteristic changes in GTIs and chronic cervical inflammation due to immunoaging (5, 12). Owing to the immunosuppressive effects of HPV, the local anti-tumor immune activity of cervical cancer usually becomes defective. CVMs tend to have anti-inflammatory effects in older women with persistent HPV infections (13). From an aging perspective, from 20–49 years, an increase in bacterial vaginosis may result from decreased estrogen levels, reduced glycogen content, and increased vaginal pH (14); in addition, a decrease in bacterial vaginosis after the age of 60 may result from a decreased immune response to infection and sexual inactivity that can occur with aging (15). A previous study on age distribution of vulvovaginal candidiasis similar to our results, the infection rate of vulvovaginal candidiasis decreased from 5.24% to 0.90% from the age of 20 years to over 65 years (15). A previous study on the age distribution of vulvovaginal candidiasis (aged 57–85, $n = 1{,}016$) found that the odds of detecting vulvovaginal candidiasis decreased by 7% each year after the age of 57 and was the lowest (0.96%) in the oldest 85-year group (15). Similarly, several studies have found that the effect of age on GTIs varies greatly before and after menopause. Menopause is associated with estrogen loss, which affects CMV metabolism, immune balance, a shift between Th1/Th2 immune adaptive responses, vaginal atrophy, reduced abundance of lactobacilli, and increased abundance of other bacterial species (16, 17). However, in addition to the effect of age on the CVM leading to GTIs, other confounding factors affect the risk of GTIs such as frequency of intercourse, douching, pessaries, and smoking status (18).

The association of vulvovaginal candidiasis and bacterial vaginosis with cervical lesions (including cytological abnormalities and CIN) has been similarly analyzed in some previous research (19). In these studies, cytological screening detected not only cytological abnormalities but also bacterial vaginosis and vulvovaginal candidiasis, and cervical dysplasia was pathologically detected as CIN. The results revealed that neither vulvovaginal candidiasis nor bacterial vaginosis was associated with cervical lesions (10, 11). Similar to the results of the current study, neither bacterial vaginosis nor vulvovaginal candidiasis was found to be associated with an increased risk of cytologic abnormalities. However, several reports have revealed that the severity of cervical neoplasms is associated with bacterial vaginosis. One systematic review showed that bacterial vaginosis was associated with an increased risk of CIN development [OR, 1.56; 95% confidence interval (CI), 1.21–2.00; $P < 0.05$], while vulvovaginal candidiasis and CIN were not significantly associated (OR 0.99; 95% CI 0.50–1.98, $P = 0.98$) (20). In contrast to the cytology smears in the current study, another study of 1,976 Pap smears, in which clue cells represented bacterial vaginosis, found that the relative risk for CIN III/CIS was 5.0 with bacterial vaginosis ($n = 9$) compared to the absence of bacterial vaginosis ($n = 16$; ref. 21). However, that study did not include a control group for the presence of HPV or vulvovaginal candidiasis. Moreover, a separate clinical study of 588 women identified a significant correlation between bacterial vaginosis and CIN, regardless of CIN severity. The incidence of CIN was significantly higher in the bacterial vaginosis-present group than in the bacterial vaginosis-absent group (94.6% vs. 81.8%, $P = 0.043$); however, the study did not test for HPV. Therefore, it was not clear whether bacterial vaginosis was the driving factor compared with HPV coinfection. Finally, a multivariate analysis using logistic regression revealed no statistical significance ($P = 0.081$; ref. 9).

In the current study, neither bacterial vaginosis nor vulvovaginal candidiasis coinfection with HPV was found to be associated with an increased risk of cytologic abnormalities compared with HPV infection alone. Some studies have suggested that no significant association exists between the presence of bacterial vaginosis and HPV infection in cervical lesions (9, 22, 23). As for the association between bacterial vaginosis (sampling by vaginal swabs) and HPV infections, a previous study involving 588 women with abnormal Pap smears who had undergone a loop electrosurgical excision procedure (LEEP) showed that bacterial vaginosis with HPV coinfection had no significant association with CIN ($P = 0.873$; ref. 9). However, other studies have reported different findings. One study suggested that bacterial vaginosis (OR,3.54; 95% CI, 1.62–7.73) was associated with the severity of CIN in HPV-positive women (24), which suggests that the microenvironment is related to the natural history of cervical neoplasia.

The role of chronic inflammation involves the participation of cytokines, chemokines, cell growth and survival factors, reactive oxygen/nitrogen species, and other mediators, including metalloproteinase, prostaglandins, COX_2, and

specific types of miRNAs (5, 6, 13, 14, 16, 17). The collective effects of these mediators promote alterations in cell proliferation, death, senescence, mutations, and angiogenesis. All these factors may contribute to the progression of HPV-induced cervical cancer (6, 7, 13). Thus, the interplay between HPV-infected host cells and the local CVM may determine the course of cervical carcinogenesis. The microbiome in the local CVM can modulate immune responses, including critical components of antitumor immunity, thereby contributing to the therapeutic activity of immune checkpoint inhibitors (12). However, crosstalk between the local vaginal microbiome and the CVM during cervical carcinogenesis remains controversial. The CVM may play a vital role in the regulation of HPV infection by evading immune surveillance with a balance between proinflammatory and antiinflammatory states (5, 12, 25). Several reports have illuminated the functional interplay between HPV, host defense mechanisms, and the CVM in cervical carcinogenesis (24, 26). Notably, one study demonstrated that GTIs changed in the CVM in cervical neoplasia, depending on genital inflammation and vaginal microbiota composition (27). bacterial vaginosis and vulvovaginal candidiasis are the most common GTIs and can cause major changes in the CVM. Furthermore, the degradation of innate defenses may occur (5, 25). In addition, persistent chronic inflammation may lead to anti-inflammatory effects and regulate the expression of cytokines, such as IL6, which may increase the risk of cytologic abnormalities (12). In previous research by the authors of the current study, it was observed that chronic inflammation (graded by inflammatory cell count) significantly increased the risk of cytologic abnormalities (28). However, in this study, vulvovaginal candidiasis and bacterial vaginosis were not found to increase the risk of cytologic abnormalities, which may also be related to the influence of immune function and inflammatory states. When acute GTIs such as bacterial vaginosis occur, the CVM tends to be proinflammatory, which produces nonspecific protective antimicrobial oxidants. Thus, it is more conducive to eliminating HPV and correlates with fewer cytologic abnormalities (5, 25). Patients with bacterial vaginosis and HPV coinfection tend to have symptoms of acute vaginitis, and metronidazole drugs are required to treat bacterial vaginosis according to clinical norms. However, this study found that bacterial vaginosis may protect women with HPV infection from cytologic abnormalities, suggesting a potential strategy to promote HPV clearance by developing bacterial biologics with bacterial-like antigenics to activate immune responses.

As the HPV vaccination status of participants was not obtained during the study, it could only be analyzed based on vaccination data from National Health Commission of the People's Republic of China for Guangzhou during the same time period. The coverage rate of the HPV vaccine in Guangzhou between 2018 and 2020 was very low at approximately 1.50% (29, 30). The low coverage rate was mainly due to low rate of awareness, high cost, and low production capacity for the HPV vaccine, as well as an insufficient supply of nine-valent vaccines (29, 30, 31). Thus, of the 14,679 patients included in this study, it is estimated that approximately 220 may have been vaccinated. According to data on the efficacy of the vaccine against cytologic abnormalities, the protective power of CIN2+ was approximately 0.11 for less than 1,000 women (31). On the basis of these data, CIN2+ among 220 women was almost 24; therefore, the mild interference effect on the results of this study might be disregarded. When the HPV vaccine has a wider coverage rate in China in the future, it can be further compared with current data to understand the preventive effect of the vaccine.

This study had some limitations. First, it included a cross-sectional study design, which allowed for demonstrating correlations but not causal correlations. Future studies with longitudinal designs are needed to extend the findings on causation relationships. Second, this study analyzed the correlation between bacterial vaginosis or vulvovaginal candidiasis and cytologic abnormalities, but cases of bacterial vaginosis coinfection with vulvovaginal candidiasis were excluded from this study. With more cases, future studies should explore the effect of bacterial vaginosis coinfection with vulvovaginal candidiasis on cytologic abnormalities. Third, mechanistic studies using *in vitro* and *in vivo* models are required to elucidate the biological mechanisms underlying the complex interplay between the influence of host immunity by vulvovaginal candidiasis or bacterial vaginosis coinfection with HPV and cytologic abnormalities. Further studies are needed to explore whether researchers can adjust the inflammatory balance in CVM using proinflammatory cytokines, promote HPV clearance, and suppress the development of cytologic abnormalities.

Conclusion

Neither vulvovaginal candidiasis nor bacterial vaginosis was found to increase the incidence of cytologic abnormalities regardless of the presence or absence of HPV. On the contrary, bacterial vaginosis may play a role in preventing cytologic abnormalities in women with HPV coinfection.

Authors' Disclosures

No disclosures were reported.

Authors' Contributions

T. Long: Formal analysis, investigation, methodology, writing–original draft, writing–review and editing. **C. Zhang:** Formal analysis, writing–original draft, writing–review and editing. **G. He:** Resources, data curation. **Y. Hu:** Resources, data curation, formal analysis. **Z. Lin:** Supervision, funding acquisition, writing–review and editing. **L. Long:** Supervision, funding acquisition, writing–review and editing.

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Note

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