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AN INVESTIGATION THE RELATIONSHIP OF VAGINAL MICROBIAL BACTERIA SPECIES WITH BACTERIAL VAGINOSIS AND HIGH-RISK HUMAN PAPILLOMAVIRUS

VAJİNAL MİKROBİYAL BAKTERİ TÜRLERİNİN, BAKTERİYEL VAJİNOZİS VE YÜKSEK RİSKLİ İNSAN PAPİLLOMAVİRUS İLE İLİŞKİSİNİN ARAŞTIRILMASI

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Abstract

Objective: This study was aim to investigate the relationship of vaginal bacterial species with High-Risk Human Papillomavirus (HR-HPV) and bacterial vaginosis (BV).
Methods: One hundred and twenty-one women were included in the study. Gram stain was performed for the diagnosis of BV and evaluated according to the Nugent score. HR-HPV diagnosis was made by real-time PCR . Detection of vaginal microbial species and *Gardnerella vaginalis* subtypes were also performed by real-time PCR.
Results: The prevalence of BV was found as 38.8%. The mean number of species was found significantly higher in BV-positive samples compared to BV-intermediate and BV-negative samples (*p=0.001*). *Lactobacillus iners* (*p*=0.036), *BVAB2* (*p*=0.043), *Provetella spp*. (*p*=0.015), *Leptotrichia/Sneathia* (*p*=0.001), *Megaspheara* (p=0.048) were found to be associated with bacterial vaginosis. *Gardnerella vaginalis* subtypes were evaluated in 50 randomly selected samples. The most common strain that was found was "clade 4". The prevalence of HR-HPV was 9.9%. HPV 16 was the most common HR-HPV type (58.3%). There was no significant difference between the mean value of *Lactobacillus* sp. HR-HPV-positive and negative samples (*p*=0.23). No association was found between the specified species and HR-HPV-positive samples (*p*=0.436).
Conclusion: Bacterial diversity was greater in BV-positive patients and BV was significantly associated with *Lactobacillus iners, Megaspheara, BVAB2, Provetella spp*. and *Leptotrichia / Sneathia*.

Keywords: *Bacterial vaginosis, Gardnerella vaginalis, human papillomavirus, vaginal microbial species.*

Öz

Amaç: Bu çalışmada vajinal mikrobiyal bakteri türlerinin bakteriyel vajinozis (BV) ve yüksek riskli Human papillomavirus (HR-HPV) ile ilişkisinin araştırılması amaçlanmıştır.
Yöntem: Çalışmaya 121 kadın dahil edilmiştir. BV tanısı için Gram boyama yapıldı ve Nugent skoruna göre değerlendirildi. HR-HPV tespiti Real Time High Risk HPV kiti kullanılarak real-time PCR yöntemiyle gerçekleştirildi. Vajinal mikrobiyal türler ve *Gardnerella vaginalis* subtipleri de real-time PCR ile saptanmıştır**.**
Bulgular: Bakteriyel vajinozis görülme oranı %38,8 olarak bulunmuştur. Tür sayısı ortalaması; bakteriyel vajinoziste, BV ara değer ve BV negatif örneklere göre anlamlı olarak yüksek görülmüştür. (*p=0,001*). *BVAB2* (*p*=0,043), *Provetella spp*. (p=0.015), *Leptotrichia/Sneathia* (*p*=0,001), *Megaspheara* (*p*=0,048), BV ile ilişkili olduğu görülmüştür. Rastgele seçilen 50 örnekte *G.vaginalis* subtipleri değerlendirilmiştir. En yaygın "clade 4" subtipi tespit edilmiştir. HR-HPV prevalansı %9,9 olarak saptanmıştır. En sık saptanan HR-HPV tipi HPV 16 %58,33'dü. HR-HPV pozitif ve negatif hastalarda Lactobacillus sp. ortalaması arasında anlamlı fark görülmemiştir (*p*=0.23). Belirlenen türler ile HR-HPV pozitifliği arasında ilişki saptanmamıştır (*p*=0,436).
Sonuç: BV tespit edilen kadınların vajenlerinde bakteri çeşitliliği daha fazlaydı. Bakteriyal vajinozis ile *Megaspheara, BVAB2, Provetella spp*. ve *Leptotrichia/Sneathia'nın* ilişkili olduğu bulunmuştur.

Anahtar Kelimeler: *Bakteriyel vajinozis, insan papillomavirus, Gardnerella vaginalis, vaginal microbiyal türler.*

Introduction

It is known that a diverse number of microorganisms, most of which are bacterial species, form the microbiota in various parts of the human body in colonies. The vagina, which is one of the important human microbiota habitats, hosts a great number of bacterial species that are important to the health of the host. The changes in the types and rates of microbial species in the vagina may cause disease.[1]

Using molecular-based techniques, it has been found that a limited number of *Lactobacillus* species such as *Lactobacillus iners, L. crispatus, L. jensenii* and *L. gasseri* dominate the normal vaginal flora.[2]

Bacterial vaginosis (BV), considered to be the most common vaginal disorder in women of reproductive age, is characterized by a large decrease in *Lactobacilli* and a high concentration of other anaerobic and facultative anaerobic bacteria replacing the normal vaginal flora. While some women with bacterial vaginosis present with vaginal discharge and unpleasant vaginal odor, others with the same microbiota are asymptomatic.[3] The persistent of genital Human Papillomavirus (HPV) infection in humans can cause cancer and the persistence was shown to be associated with many factors.[4]

There is strong evidence that the native bacterial communities living in the vagina serve as an important line of defense against non-vaginal pathogens, including sexually transmitted deseases (STDs).[5] This study was aim to determine the type of vaginal bacteria concerning BV and to investigate the relationship of bacterial species with High-Risk Human Papillomavirus (HR-HPV) and BV.

Methods

Patient Group

One hundred and twenty-one women who applied to Pamukkale University Hospital of Obstetrics and Gynecology outpatient clinic between March 2017 and October 2017 were included in the study. Their cervical and vaginal swab samples were collected during the clinical visits by the clinical staff. For all the samples, the collected swabs were immediately put inside Stuart transport medium and Cervi-Collect Specimen Collection Kit Transport Tube (Abbott, USA). Women under the age of 18, immunocompromised patients, those who received antibiotic treatment within the last month, those who used vaginal anti-inflammatory agents, antihistamine drugs and those who used intrauterine devices were not included in the study. Samples were stored at +4 °C for 3 days to be used in HR-HPV studies. Samples were stored at -20 °C to be used in the studies of vaginal microbial species.

All participation was voluntary and anonymized, and written informed consent was obtained. This study was performed according to ethical permission approved by Pamukkale University Ethics Committee (10.01.2017/01).

BV Diagnosis

A vaginal swab sample taken for the diagnosis of BV was spread on the slide and Gram stain was performed. It was assessed using the Nugent score. If the total score of smears was between 7-10, BV was considered positive, smears of 4-6 points were accepted as intermediate value, and smears of 0-3 were considered negative for BV.

HR-HPV Detection

Patient samples were taken out of the +4°C refrigerator. In the ABBOTT m2000sp isolation device, the extraction steps and the master mix addition steps were carried out in line with the company's recommendations.

Detection of Vaginal Microbial Species

The detection of vaginal microbial species was performed by real-time PCR. For DNA isolation; samples were obtained from "Cultiplast swabs", whose vaginal swab samples were taken using a genomic DNA isolation kit (RTA, RTA Laboratories, Turkey). DNA extraction was performed according to the manufacturer's recommendations. LightCycler™ system and "LightCycler Faststart DNA Master SYBR Green I" were used with DNA primers. Primers were selected from the published article.[6]

Detection of *Gardnerella vaginalis* clades

Detection of *G. vaginalis* "clades" was done by real-time PCR. DNA isolation was obtained from "Cultiplast swabs" from which vaginal swab samples were taken with "Roche® High Pure PCR Template Preparation kit". The DNAs obtained were studied with the Roche LightCycler 480 II device using the LightCycler® 480 SYBR Green I Master with primers. Primers were selected from the published article.[7]

Statistical Analysis

The statistical analysis of data was carried out by using SPSS 24 (IL, USA). Continuous variables are expressed as mean±standard deviation, minimum-maximum values and categorical variables are given in numbers and percentages. Differences between categorical variables were examined by chi-square analysis. For the comparison of independent groups, the assumptions were checked and Mann-Whitney U-test, Kruskal-Wallis variance analysis (post hoc: Mann-Whitney U-test with Bonferroni correction) and one-way variance analysis (post hoc: Tukey test) were used. The kappa analysis was used to evaluate compliance. Logistic regression analysis was used to analyze risk factors. $p<0.05$ was taken to indicate statistical significance in all analyses.

Results

One hundred and twenty-one women were evaluated for bacterial vaginosis, HR-HPV and vaginal microbial species. *G. vaginalis* subtypes were evaluated in 50 individuals randomly selected among the participants. Of the participants, 55 (66.6%) were symptomatic patients and 66 (33.4%) were asymptomatic patients. Of the samples evaluated according to Nugent score for BV diagnosis, 48 were BV-negative (39.7%), 26 were BV-intermediate (21%), 47 were BV-positive (38.8%). BV positivity was mostly observed in the 3rd and 4th decades.

A statistically significant difference was found between menstrual periods of individuals according to BV, BV-negative, BV-intermediate status ($p=0.019$). An increase in BV positivity was detected in the secretory phase of the menstrual cycle. Based on the classification as BV-negative, BV-intermediate and BV-positive status, a statistically significant difference was found in terms of the last time they had sexual intercourse (within 1-3 days, within 3-7 days, and more than 7 days before hospital admission) ($p=0.027$). The prevalence of BV was higher in individuals who had sexual

intercourse within 1-3 days before admission to the hospital (29.3%). Five women who desired to be pregnant and 47 menopausal women had no protection during sexual intercourse. A statistically significant difference was found between protected and unprotected individuals during sexual intercourse according to their BV-negative, BV-intermediate and BV-positive status (p=0.002). BV negativity was very high in those who were protected during sexual intercourse (72.9%). Although there was no statistically significant difference between BV-negative, BV-intermediate and BV-positive status based on the protective methods used, BV negativity was higher in individuals using condoms compared to the other groups (42.9%) (Table 1).

Excluding *Lactobacillus* species (*Megaspheara, G. vaginalis, BVAB2, Mobilincus mulieris, Provetella spp., Leptotrichia/Sneathia*), the average number of species investigated was 1.36±0.7 (1-4) in BV-negative samples, 1.46±0.81 (1-3) in intermediate samples, and BV-positive samples were found to be 2.28±1.48 (1-6). According to post hoc examination, the mean number of species was significantly higher in BV-positive samples than BV-negative and BV-intermediate samples (p=0.001). *Lactobacillus iners* was commonly found in healthy, BV-intermediate women and BV-positive patients (62.50%, 50.00%, 78.72%, respectively). *L. iners* was statistically significantly higher in BV-positive samples than BV-intermediate and BV-negative samples (p=0.036). *L. iners* was not evaluated quantitatively.

Megaspheara (p=0.048), *Lactobacillus iners* (p=0.036), *Provetella spp*. (p=0.015) *Leptotrichia/Sneathia* (p=0.001) and *BVAB2* (p=0.043) were significantly higher in BV-positive samples than in BV-intermediate and BV-negative ones (Table 1). Several bacterial groups associated with BV were strongly related to each other. The simultaneous detection of *Provetella spp.-Leptotrichia/Sneathia*, *Provetella spp-BVAB2*, *Provetella spp.-Megaspheara* was highly significant (p<0.001) (Table 2).

Table 1. Variable distribution of BV negative, BV intermediate and BV positive n (%)

	BV negative	**BV intermediate**	**BV positive**	***p* value**
Marital status				
Married	43 (89.6)	18 (69.2)	40 (85.1)	0.092
Single	5 (10.4)	8 (30.8)	7 (14.9)	
Smoke				
Yes	12 (25.5)	4 (15.4)	7 (14.9)	0.371
No	35 (74.5)	22 (84.6)	40 (85.1)	
Menstrual cycle				
MP+PP+O	13 (27.1)	3 (11.5)	12 (25.5)	
SP	23 (47.9)	6 (23.1)	17 (36.2)	0.019*
Menopause	12 (25)	17 (65.4)	18 (38.3)	
Vaginal Shower+Gel				
Yes	-	-	-	-
No	48 (100)	26 (100)	47 (100)	
Last intercourse date				
1-3 days ago	7 (16.3)	3 (18.8)	12 (29.3)	
3-7 days ago	13 (30.2)	2 (12.5)	12 (29.3)	0.027*
More than 7 days	23 (53.5)	11 (68.8)	17 (41.4)	
Drug use				
Yes	6 (12.5)	5 (19.2)	4 (8.5)	0.427
No	42 (87.5)	21 (80.8)	43 (91.5)	
Protected				
Yes	35 (72.9)	8 (30.8)	26 (55.3)	0.002*
No	13 (27.1)	18 (69.2)	21 (44.7)	
Protected Metod				
Condom	15 (42.9)	1 (12.5)	6 (23.1)	0.107
Other protected	20 (57.1)	7 (87.5)	20 (76.9)	

MP: Menstrual Phase, PP: Proliferative Phase, O: ovulation, SP: Secretory Phase, *p<0.05

Table 2. Relation of bacteria determined by PCR with BV negative, BV intermediate and BV positive samples n (%)

Bacteria	BV negative	BV intermediate	BV positive	*p* value
Lactobacillus crispatus	3 (6.3)	3 (11.5)	3 (6.4)	0.667
Lactobacillus iners	30 (62.5)	13 (50.0)	37 (78.7)	0.036
Lactobacillus gasseri	16 (33.3)	5 (19.2)	19 (40.4)	0.183
Lactobacillus jensenii	18 (37.5)	8 (30.8)	26 (55.3)	0.078
Garnerella vaginalis	47 (100.0)	26 (100.0)	47 (100.0)	–
Megaspheara	10 (20.8)	6 (23.1)	20 (42.6)	0.048
BVAB2	1 (2.1)	1 (3.8)	7 (14.9)	0.043
Leptotrichia/Sneathia	3 (6.3)	2 (7.7)	16 (34.0)	0.001
Provetella spp.	4 (8.3)	3 (11.5)	14 (29.8)	0.015
Mobilincus mulieris	0 (0.0)	0 (0.0)	3 (6.4)	0.089

–: Not tested as it is 100%

Of the 50 subjects studied with *G. vaginalis* subtypes, 12 were BV-positive (24%), 20 were BV-intermediate (40%) and 18 were BV-negative (36%). The most common strain that was found was "clade 4". There was no significant relationship between the Clades of BV ($p>0.05$). Albeit not significant, association with multiple "clades" was 3 times higher than association with a single "clade" in BV-positive samples. An examination of the relationship of *G. vaginalis* subtypes with BV-negative, BV-intermediate and BV-positive samples presented no significant difference ($p>0.05$) (Table 3).

In our study, the incidence rate of HR-HPV was found to be 9.9%. The highest HR-HPV positivity was observed in the 3rd and 5th decades. HPV 16 (n=7) was found to be the most common HR-HPV type (58.3%). Other high-risk groups were determined to be 33.3% (n=4) and HPV 18 was 8.3% (n=1).

Marital status was found to be a significant risk factor among the groups (OR=4.48, $p=0.021$). Unmarried individuals were 4.48 times more likely to be HR-HPV-positive than those who were married (Table 4). When the association between the mean number of vaginal microbial species determined by PCR and HR-HPV positivity was evaluated, no difference was found. Also, an evaluation of the association between PCR-determined species and HR-HPV positivity showed no significant difference ($p>0.05$).

There was no significant difference in terms of the association between HR-HPV positivity and BV, BV-negative, BV-intermediate groups ($p=0.525$). Albeit not significant, the negativity of HR-HPV in the BV-negative (93%) group was found to be higher than the BV-intermediate (88.5%) and BV-positive (87.2%) groups.

Table 3. Relationship of *G. vaginalis* subtypes with BV negative, BV intermediate and BV positive samples n (%)

	BV negative	BV intermediate	BV positive	*p* value
Clade 1				
Negative	9 (50)	12 (60)	5 (41.7)	0.59
Positive	9 (50)	8 (40)	7 (58.3)	
Clade 2				
Negative	12 (66.7)	12 (60)	6 (50)	0.66
Positive	6 (33.3)	8 (40)	6 (50)	
Clade 3				
Negative	15 (88.3)	10 (50)	9 (75)	0.073
Positive	3 (16.7)	10 (50)	3 (25)	
Clade 4				
Negative	5 (27.8)	6 (30)	3 (25)	0.954
Positive	13 (72.2)	14 (70)	9 (75)	

Table 4. Variable distribution of HPV negative and HPV positive individuals n (%)

	HPV negative	**HPV positive**	***p* value**
Marital status			
Married	94 (86.2)	7 (58.3)	0.021*
Single	15 (13.8)	5 (41.7)	
Smoke			
Yes	20 (18.5)	3 (25)	0.698
No	88 (81.5)	9 (75)	
Menstrual cycle			
MP+PP+O	25 (22.9)	3 (25)	
SP	44 (40.4)	2 (16.7)	0.207
Menopause	40 (36.7)	7 (58.3)	
Vaginal Shower+Gel			
Yes	-	-	-
No	109 (100)	12 (100)	
Last intercourse date			
1-3 days ago	21 (22.8)	1 (12.5)	
3-7 days ago	24 (26.1)	3 (37.5)	0.691
More than 7 days	47 (51.1)	4 (50)	
Drug use			
Yes	13 (11.9)	2 (16.7)	0.644
No	96 (88.1)	10 (83.3)	
Protected			
Yes	64 (58.7)	5 (41.7)	0.258
No	45 (41.3)	7 (58.3)	
Protected metod			
Condom	19 (29.7)	3 (60)	0.318
Other protected	45 (70.3)	2 (40)	

MP: Menstrual Phase, PP: Proliferative Phase, O: ovulation, SP: Secretory Phase, *$p<0.05$

Discussion

BV is an ecological disorder of the vaginal microbiota that affects millions of women each year.[8] BV prevalence was found to be 38.8% in our study. Studies have shown that there are differences between BV prevalence according to geographical locations. The prevalence of BV in South Africa was 44%; in a study conducted with 163 women in Sweden, the prevalence of BV was found to be 44.7%, and in a study including 264 women in Seattle, the prevalence of BV was 30.7%. In the study conducted by Li et al. with a large population of 53,652 married women of reproductive age in Anhui province of China, the prevalence of BV was found to be 11.9%.[6,9-11] The differences in prevalence of BV may be due to geographic regional differences, the size of the population included in the studies, the socioeconomic levels, and the private behavior of the individuals.

In our study, the prevalence of BV was observed at a high rate of in the age range of 31-40 and in the age range of 41-50. Ranjit et al. Reported similar findings in their study. The reason why BV is observed more frequently in the 31-50 age group may be due to the fact that these age groups are sexually active and they have a longer history of sexual intercourse than younger individuals.[12] An increase in BV positivity was detected in the secretory phase of the menstrual cycle. During the progesterone-high secretory phase of the menstrual cycle, the cytotoxic T lymphocyte activity and natural killer cell cytotoxic activity in the uterus are suppressed, while the innate defense system is increased. The resulting immune changes create a vulnerability for sexually transmitted infections and may increase the risk of acquiring these diseases.[13]

In our study, the prevalence of BV was higher in people who had sexual intercourse 1-3 days before admission to the hospital. Bautista et al. conducted a literature review in their study, and in several studies over the past decade, they found evidence that sexual activity contributes to the development of BV. However, they concluded that it is difficult to state that BV is a sexually transmitted disease without identifying its etiological agent. [14] According to the study conducted by Vodstrcil et al. regarding the influence of sexual activity on the vaginal microbiota and *G. vaginalis* clade diversity in young women, it was concluded that sexual activity does influence the composition of vaginal microbiota in young women who are sexually inexperienced and penile vaginal sex does not change the consistency of the microbial community; however, there was increased *G. vaginalis* clade diversity in young women irrespective of their BV status. This suggests sexual transmission of commensal and potentially pathogenic clades of *G. vaginalis*.[15] In our study, BV positivity was significantly different between protected and unprotected individuals during sexual intercourse. It is a result of the lack of use of pelvic intrauterine devices by the participants in our study, and the methods they chose to use for prevention are methods to reduce the risk of BV. BV negativity is very high in those who were protected during intercourse.

Bacterial vaginosis is caused by the transition to a heterogeneous anaerobic and/or aerobic bacterial community as a result of decreased *Lactobacillus* dominance in the vaginal flora.[16] In our study, the abundance of non-*Lactobacillus* (*Megaspheara, G. vaginalis, Leptotrichia / Sneathia, BVAB2, M. mulieris, Provetella spp.*) species was found to be higher in BV samples compared to BV-negative and BV-intermediate samples. Women with BV had heterogeneous communities of vaginal bacteria. Our findings were compatible with those reported in the literature.[6,10,17]

In our study, *L. iners* was found to be common in healthy, BV-intermediate and BV-positive patients. It was significantly higher in BV-positive samples than BV-intermediate and BV-negative samples. In our study, *L. iners* burden was not evaluated quantitatively. Zozaya et al. showed that the abundance of *L. iners* is high in all categories, including BV patients.[18] Datcu et al. also detected *L. iners* in all of the participants in their study.[19] Although *M. mulieris* was not statistically significant, this detection was higher in BV-positive samples that was compatible with other studies.[1,6,10,17,19]

G.vaginalis is strongly associated with BV. It is one of the most common bacteria detected in women with symptoms of BV.[20] Recent studies have shown that *G. vaginalis* may be a part of vaginal microbiota in clinically healthy women.[21,22] In our study, *G. vaginalis* was found in 100% of BV-negative women. In a study conducted by Janulaitiene et al. with 109 Lithuanian women, *G. vaginalis* was found in 87% of BV-negative women.[17] Balashov et al. found *G. vaginalis* in 97% of BV-negative women in their study, in which they analyzed *G. vaginalis* bacterial load and "clade" distribution.[7] The detection of *G. vaginalis* in all samples can be explained by the fact that PCR detects even low levels of microorganisms. *G. vaginalis* burden was not evaluated quantitatively in our study. Our findings are consistent with the *G. vaginalis* percentages seen in the literature.[7,17] *G.vaginalis* is divided into four different clades (1-4) and four corresponding subgroups (A-D).[7] In our study, *G. vaginalis* "clade 4" was found to be the most common "clade". Similarly, in other studies, "clade 4" was found to be the most prevalent "clade".[7,17] In our study, there were multiple clade associations in different combinations. Multiple clades were found in 66% of the samples. Similar to our study, Balashov et al.[7] detected multiple "clades" in 70% of their samples.

In our study, there was no association between the existence of single or multiple clades and BV. Vodstrcil et al., in their study investigating the effects of sexual activity of young women on vaginal microbiota and *G. vaginalis* "clade" diversity, found that the presence of multiple "clades" had a positive association with BV.[15] In our study, in BV-positive samples, association with multiple "clades" was 3 times higher than association with a single "clade". When the relationship between *G. vaginalis* "clades" and BV-negative, BV-intermediate and BV-positive samples was examined, no significant difference was found. Aroutcheva et al. concluded that a specific phenotype or genotype of *G. vaginalis* does not cause BV.[23] Similat to our study, Tosun et al. also did not report a significant difference between the biotype distribution of BV patients and those with negative BV (p=0.687).[21]

In our study, the rate of HR-HPV was %9.9. HPV 16 was found to be the most common HR-HPV type. Our findings were consistent with the literature.[24-26] Our study showed that HR-HPV-positivity had its first peak at the age of 30 that the rate decreased to at the age of 40, and a second peak is observed between the ages of 51 and 60. Our findings are compatible with those reported in the literature.[25] Similar to a previous study,[27] in our study, unmarried individuals were 4.48 times more likely to be HR-HPV-positive than those who were married. It should also be taken into account that singles can have multiple partners. However, this situation was not questioned in the study.

In our study, there was no significant difference between the mean of *Lactobacillus* sp. in HR-HPV-negative and HR-HPV-positive samples. When *Lactobacillus* sp. were excluded, there was no significant difference between the means of bacterial species studied. This was not consistent with the literature.[28,29] In our study, the reason for the absence of a significant difference between the groups in terms of microbial diversity may be due to the analysis of a small number of HR-HPV-positive samples. When the relationship between HR-HPV-positivity and BV presence, were evaluated, no significant difference was found (p=0.525). Same result was found in previous study[30] Although no significant difference was found, the negativity of HR-HPV in the BV-negative group was found to be higher than in BV-intermediate and BV-positive group. The relationship between BV and HR-HPV infection resulted in a positive correlation in some studies, whereas other studies reported no relationship between them.[3,30,31]

The limitations of our study; diet and obesity are also risk factors for BV and they were not evaluated in this study. BV increases the risk of not only HPV but also other STDs. A future study might be planned to evaluate the relationship between BV and other STDs.

In conclusion, bacterial diversity was higher in the BV positive group in our study. *L. iners, Megaspheara, BVAB2, Provetella spp.* and *Leptotrichia/Sneathia* were found to be associated with BV. *G. vaginalis* clade 4 and the HPV 16 were the most common types. There was no relationship between HPV and BV.

Conflict of Interest

The authors declare no conflict of interest.

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Compliance with Ethical Statement

Ethical approval of this study was obtained from Pamukkale University Ethics Committee prior to initiation of the research work (10.01.2017/01).

Author Contributions

SZÖ, İK, BK: The hypothesis of the study; SZÖ, İK, BK: Study desing; SZÖ, İK, BK: Project development; SZÖ, İK, BK: Literature search; SZÖ: Analysis; SZÖ: Manuscript writing; SZÖ, İK, BK: Critical review

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