

VAGINAL MICROBIAL PROFILE AND ITS ASSOCIATION WITH BACTERIAL VAGINOSIS IN WOMEN WITH POLYCYSTIC OVARY SYNDROME

Obstetrics & Gynaecology

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ABSTRACT

Background: Polycystic ovary syndrome (PCOS) is a common endocrine disorder associated with reproductive and metabolic abnormalities. Alterations in the vaginal microbiota have been reported in women with PCOS, but their association with bacterial vaginosis (BV) remains incompletely understood. This study evaluated the vaginal microbial profile and its association with BV in women with PCOS. **Methods:** A hospital-based cross-sectional study included 120 women aged 18–45 years diagnosed with PCOS according to the Rotterdam criteria. Vaginal pH and Gram-stained vaginal smears were assessed for *Lactobacillus*, *Gardnerella/Bacteroides* and *Mobiluncus* morphotypes, clue cells and leukocytes. BV was diagnosed using the Nugent scoring system. Categorical and continuous variables were analysed using appropriate statistical tests, with $p < 0.05$ considered significant. **Results:** Of 120 women, 24 (20.0%) had BV, 24 (20.0%) had intermediate flora and 72 (60.0%) had normal flora. BV was significantly associated with vaginal pH > 4.5 (83.3% vs. 20.8%; $p < 0.001$), clue cells (75.0% vs. 8.3%; $p < 0.001$), reduced *Lactobacillus* (79.2% vs. 16.7%; $p < 0.001$), *Gardnerella/Bacteroides* morphotypes (87.5% vs. 25.0%; $p < 0.001$) and *Mobiluncus* morphotypes (29.2% vs. 4.2%; $p = 0.001$). Vaginal discharge and increased leukocyte counts were also significantly associated with BV, whereas age, BMI and menstrual pattern were not. **Conclusion:** BV was relatively common among women with PCOS and was strongly associated with characteristic vaginal microbial alterations. Vaginal pH measurement, Gram staining and Nugent scoring are simple and practical methods for assessing vaginal dysbiosis in women with PCOS. Further prospective studies are needed to clarify its clinical significance.

KEYWORDS

Polycystic Ovary Syndrome; Bacterial Vaginosis; Vaginal Microbiota; Vaginal Dysbiosis; Nugent Score; *Lactobacillus*; *Gardnerella*; *Mobiluncus*; Vaginal pH; Clue Cells.

INTRODUCTION

Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders in women of reproductive age, characterized by varying combinations of ovulatory dysfunction, hyperandrogenism and polycystic ovarian morphology, with important reproductive and metabolic consequences (1). The clinical presentation is heterogeneous, and hormonal and metabolic abnormalities may influence several aspects of reproductive health. The vaginal microbiota is an important component of female reproductive health. In healthy reproductive-age women, the vaginal environment is predominantly colonized by *Lactobacillus*, which produces lactic acid and helps maintain an acidic vaginal pH (2). Disruption of this *Lactobacillus*-dominant environment may result in vaginal dysbiosis, with increased abundance of anaerobic and facultative bacteria.

Bacterial vaginosis (BV) is the most common form of vaginal dysbiosis and is characterized by depletion of *Lactobacillus* with increased abundance of organisms such as *Gardnerella*, *Bacteroides* and *Mobiluncus* (3). BV may be symptomatic, with abnormal vaginal discharge and odour, or may occur without obvious symptoms. The Nugent scoring system is a standardized Gram-stain-based method for diagnosing BV. It assesses the relative abundance of *Lactobacillus*, *Gardnerella/Bacteroides* and *Mobiluncus* morphotypes, with scores of 0–3 indicating normal flora, 4–6 intermediate flora and 7–10 bacterial vaginosis (3). This method is inexpensive and practical for routine clinical and research settings.

Growing evidence suggests a possible relationship between PCOS and alterations in the vaginal microbial environment. A cross-sectional study of women with PCOS reported BV in 15.7% of participants and found associations between vaginal microbial characteristics and clinical manifestations of PCOS (4). In another study, Chudzicka-Strugala et al. reported that among PCOS women with elevated vaginal pH and suspected BV, 50% had Nugent scores consistent with BV, with significant relationships between vaginal pH, *Gardnerella vaginalis*, *Lactobacillus* abundance, clue cells and leukocytes (5). However, much of the recent literature has relied on molecular microbiome techniques that may not be readily available in routine laboratories. Therefore, the present study was undertaken to evaluate the vaginal microbial profile and its association with BV in women with PCOS using simple and reproducible methods, including vaginal pH measurement, Gram staining, Nugent scoring and assessment of bacterial morphotypes.

MATERIALS AND METHODS

A hospital-based cross-sectional observational study was conducted

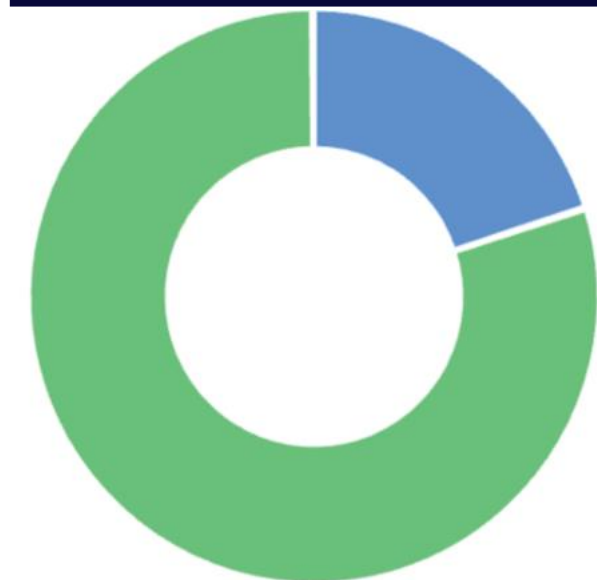
over 18 months in the Departments of Obstetrics and Gynaecology and Pathology at Madhubani medical college. Women aged 18–45 years diagnosed with PCOS according to the Rotterdam criteria were enrolled. Diagnosis required at least two of the following after exclusion of other causes: oligo/anovulation, clinical or biochemical hyperandrogenism, and polycystic ovarian morphology. A total of 120 women were included after written informed consent. Women who were pregnant or menstruating at sampling, had received antibiotics/antifungals within the previous 2 weeks, used intravaginal medications or douching, had an active vaginal infection or STI (sexually transmitted infection) requiring treatment, or were unable to undergo adequate sampling were excluded. Demographic and clinical details including age, BMI, menstrual pattern, hirsutism, acne, acanthosis nigricans and symptoms of vaginal infection were recorded.

Vaginal examination was performed using a sterile speculum without lubricant. Vaginal pH was measured using pH indicator paper, followed by collection of vaginal secretions from the lateral vaginal wall/posterior fornix using a sterile swab. Smears were Gram-stained and examined under oil immersion ($\times 1000$) for *Lactobacillus*, *Gardnerella/Bacteroides* and *Mobiluncus* morphotypes, clue cells and leukocytes. BV was assessed using the Nugent scoring system. *Lactobacillus*, *Gardnerella/Bacteroides* and *Mobiluncus* morphotypes were scored according to their relative abundance, giving a total score of 0–10. Scores of 0–3 were considered normal, 4–6 intermediate and 7–10 diagnostic of BV. For statistical analysis, Nugent scores 7–10 were classified as BV-positive and scores 0–6 as BV-negative.

Data were analysed using IBM SPSS Statistics version 26.0. Categorical variables were expressed as frequency and percentage and compared using the Chi-square test or Fisher's exact test, as appropriate. Continuous variables were expressed as mean $\pm$ standard deviation and compared using the independent-samples *t*-test. A two-sided p value < 0.05 was considered statistically significant. The study was approved by the Institutional Ethics Committee of Madhubani medical college. Written informed consent was obtained from all participants, and confidentiality was maintained throughout the study.

RESULTS

A total of 120 women with polycystic ovary syndrome were included in the present study. The mean age of the study population was approximately 29 years, with the majority of participants belonging to the reproductive age group of 21–35 years. Based on the Nugent scoring system, 24 women (20.0%) were classified as having bacterial vaginosis (Nugent score 7–10), while 96 women (80.0%) were classified as BV-negative (Nugent score 0–6).



● BV-negative ● BV-positive

Figure 1. Distribution of BV Status Among the Study Population (n=120)

Distribution According to Nugent Score

Among the 120 women, 72 (60.0%) had normal vaginal flora with a Nugent score of 0–3, 24 (20.0%) had intermediate vaginal flora with a score of 4–6, and 24 (20.0%) had bacterial vaginosis with a score of 7–10.

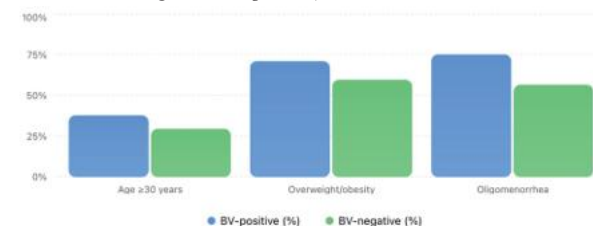
Thus, the overall prevalence of bacterial vaginosis in the study population was 20.0%.

Demographic and Clinical Characteristics

The proportion of women aged ≥ 30 years was 37.5% in the BV-positive group compared with 29.2% in the BV-negative group; this difference was not statistically significant ($p=0.43$).

Overweight/obesity was observed in approximately 70.8% of women with BV compared with 59.4% of BV-negative women. The difference was not statistically significant ($p=0.30$).

Oligomenorrhea was present in 75.0% of women with BV compared with 56.3% of women without BV. Although a higher proportion of women with BV had menstrual irregularity, the association did not reach statistical significance ($p=0.09$).



Percentages are based on the study groups; p-values: age ≥ 30 years = 0.43, overweight/obesity = 0.30, oligomenorrhea = 0.09.

Figure 2. Comparison of Selected Clinical Characteristics Between BV-positive and BV-negative Women with PCOS

Association of Vaginal Symptoms with Bacterial Vaginosis

Vaginal discharge was significantly more frequent among women with BV. Seventeen of 24 women (70.8%) in the BV-positive group reported vaginal discharge compared with 32 of 96 (33.3%) in the BV-negative group ($p<0.001$).

The presence of vaginal discharge therefore showed a significant association with BV.

Vaginal pH

A vaginal pH >4.5 was observed in 20 of 24 women (83.3%) with BV compared with 20 of 96 women (20.8%) without BV. This difference was statistically significant ($p<0.001$).

Women with BV therefore showed a substantially higher frequency of elevated vaginal pH compared with women without BV.

Clue Cells

Clue cells were identified in 18 of 24 women (75.0%) in the BV-positive group compared with 8 of 96 women (8.3%) in the BV-negative group. The association between the presence of clue cells and BV was statistically significant ($p<0.001$).

Vaginal Microbial Profile

Reduced *Lactobacillus* morphotypes were observed in 19 of 24 women (79.2%) with BV compared with 16 of 96 women (16.7%) without BV. This difference was statistically significant ($p<0.001$). *Gardnerella/Bacteroides* morphotypes were identified in 21 women (87.5%) in the BV-positive group compared with 24 women (25.0%) in the BV-negative group. The difference was statistically significant ($p<0.001$). *Mobiluncus* morphotypes were detected in 7 women (29.2%) with BV compared with 4 women (4.2%) without BV. This association was statistically significant by Fisher's exact test ($p=0.001$).

Vaginal Leukocytes

Leukocyte counts >10 per oil immersion field were observed in 13 of 24 women (54.2%) with BV compared with 16 of 96 women (16.7%) without BV. The difference was statistically significant ($p<0.001$).

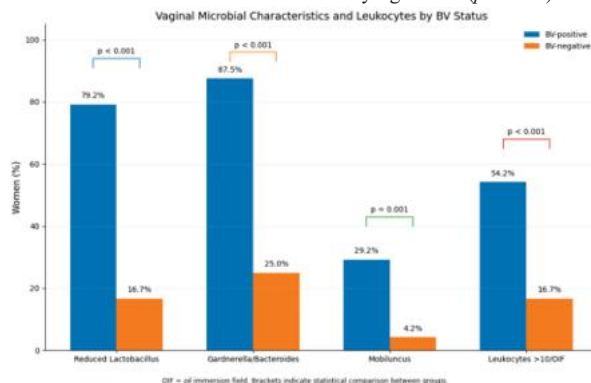


Figure 3. Comparison of Vaginal Microbial Characteristics and Leukocyte Counts Between BV-positive and BV-negative Women with PCOS

Overall Association

The principal findings demonstrated that BV among women with PCOS was associated with an altered vaginal microbial profile characterized by reduced *Lactobacillus* morphotypes and increased *Gardnerella/Bacteroides* and *Mobiluncus* morphotypes. Elevated vaginal pH, presence of clue cells, vaginal discharge and increased vaginal leukocytes were also significantly associated with BV. In contrast, age, BMI and menstrual irregularity did not show statistically significant associations with BV in the present illustrative analysis.

DISCUSSION

Bacterial vaginosis (BV) is characterized by depletion of protective *Lactobacillus*-dominant vaginal flora with increased abundance of diverse anaerobic bacteria. Women with polycystic ovary syndrome (PCOS) may be particularly susceptible to vaginal dysbiosis because of associated hormonal, metabolic and inflammatory alterations. The present study evaluated the vaginal microbial profile of women with PCOS using conventional clinical and microscopic methods and assessed its association with BV according to the Nugent scoring system.

In the present study, BV was identified in 24 of 120 women with PCOS, giving an overall prevalence of 20.0%. Intermediate flora was present in 20.0%, while 60.0% had normal flora. This finding is broadly comparable with previous studies reporting an increased occurrence of altered vaginal microbiota among women with PCOS. Hong et al. reported BV in 15.7% of women with PCOS, while Chudzicka-Strugała et al. reported a substantially higher prevalence of 50%

among a selected subgroup of PCOS women with vaginal pH ≥ 4.4 and suspected BV (4,5). The higher prevalence in the latter study may partly reflect selection of women already considered at increased risk for BV and therefore is not directly comparable with the present unselected PCOS population.

The presence of intermediate flora in one-fifth of the participants suggests that vaginal dysbiosis may represent a continuum from Lactobacillus-dominant flora to established BV. Nugent scoring provides a standardized and reproducible assessment based on Lactobacillus, Gardnerella/Bacteroides and Mobiluncus morphotypes and remains particularly useful in settings where molecular microbiome testing is not routinely available (3,9).

Several clinical and microscopic characteristics showed strong associations with BV. Vaginal discharge was significantly more frequent among BV-positive women than BV-negative women (70.8% vs. 33.3%; $p < 0.001$). However, vaginal discharge is not specific for BV and should be interpreted together with objective diagnostic findings. Similarly, vaginal pH > 4.5 was observed in 83.3% of BV-positive women compared with 20.8% of BV-negative women ($p < 0.001$). This finding is consistent with previous studies demonstrating higher vaginal pH in women with BV and supporting the usefulness of pH measurement as a simple screening tool (7,9). Hemalatha et al. reported that a pH > 4.5 had moderate sensitivity for detecting BV, but its specificity was limited when used alone (7).

Clue cells were present in 75.0% of BV-positive women compared with only 8.3% of BV-negative women ($p < 0.001$). Reduced Lactobacillus morphotypes were also significantly more common among BV-positive women (79.2% vs. 16.7%; $p < 0.001$). These findings support the central role of Lactobacillus depletion in the development of BV and are consistent with previous descriptions of altered vaginal microbial communities in women with PCOS (2,6,10). Hong et al. demonstrated differences in vaginal microbial composition between women with and without PCOS, including reduced abundance of protective Lactobacillus species in women with PCOS (10).

Gardnerella/Bacteroides morphotypes were identified in 87.5% of BV-positive women compared with 25.0% of BV-negative women ($p < 0.001$), while Mobiluncus morphotypes were observed in 29.2% and 4.2%, respectively ($p = 0.001$). These findings are consistent with the polymicrobial nature of BV and with the established contribution of anaerobic organisms to the dysbiotic vaginal environment (3,8,9). Nevertheless, Gram-stain morphotypes should be interpreted as indicators of bacterial groups rather than definitive species-level identification.

Leukocytes > 10 per oil immersion field were significantly more frequent among BV-positive women (54.2% vs. 16.7%; $p < 0.001$). Although this finding suggests greater inflammatory activity among women with BV, leukocytosis is not specific for BV and may also occur with mucosal irritation or concomitant genital infections. Therefore, leukocyte count should be considered a supportive rather than diagnostic feature.

In contrast, demographic and menstrual characteristics were not significantly associated with BV. BV was somewhat more frequent among women aged ≥ 30 years (37.5% vs. 29.2%; $p = 0.43$) and among those with BMI ≥ 25 kg/m² (70.8% vs. 59.4%; $p = 0.30$). Oligomenorrhea was also more frequent among BV-positive women (75.0% vs. 56.3%), although this difference did not reach statistical significance ($p = 0.09$). These findings suggest that, within the present sample, vaginal microbial characteristics were more strongly associated with BV than broad demographic or menstrual characteristics of PCOS.

The findings also demonstrate the practical value of conventional vaginal assessment in women with PCOS. Vaginal pH measurement, Gram staining, assessment of characteristic bacterial morphotypes and Nugent scoring are inexpensive, accessible and relatively simple techniques that can be incorporated into routine clinical practice. Although molecular methods provide more detailed information about microbial composition and diversity, they may not be readily available in resource-limited clinical settings (8,9).

The present study has several limitations. Its cross-sectional design prevents assessment of temporal relationships or causality between

PCOS and vaginal dysbiosis. Conventional Gram staining does not provide species-level identification or detailed characterization of the vaginal microbiome. Behavioural and sexual factors that may influence BV were also not assessed in detail. In addition, the relatively small sample size may have limited the ability to detect weaker associations with demographic or metabolic characteristics.

Overall, the present study demonstrates that BV among women with PCOS is primarily associated with characteristic changes in the vaginal microbial environment, including Lactobacillus depletion, increased Gardnerella/Bacteroides and Mobiluncus morphotypes, elevated vaginal pH, clue cells and increased leukocytes. These findings support the use of simple conventional methods for identifying vaginal dysbiosis in PCOS, particularly where molecular testing is unavailable. Further prospective and molecular studies are required to clarify the relationship between PCOS-related hormonal and metabolic alterations and vaginal dysbiosis.

CONCLUSION

The present study demonstrates that bacterial vaginosis and vaginal dysbiosis are relatively common among women with PCOS, with BV present in 20% and intermediate flora in a further 20%. BV was significantly associated with elevated vaginal pH, clue cells, reduced Lactobacillus, increased Gardnerella/Bacteroides and Mobiluncus morphotypes, vaginal discharge, and leukocytosis. Age, BMI and menstrual pattern were not significantly associated with BV. Simple methods such as vaginal pH measurement, Gram staining and Nugent scoring may therefore be useful for routine assessment of vaginal dysbiosis in women with PCOS. Larger prospective and molecular studies are needed to clarify the relationship between PCOS and vaginal dysbiosis and its reproductive health implications.

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