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ORIGINAL ARTICLE

Associations of First Void Urine Samples with Bacterial Vaginosis

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ABSTRACT

Objective: The aim of this study was to investigate whether BV can be diagnosed from first-void urine (FVU).
Study Design: Cross-sectional/case control study
Place and Duration: Conducted at Hameed Latif Hospital Lahore, during from Sep, 2021 to Feb, 2022.
Methods: There were 100 women had ages 20-65 years was presented in this study. Once informed written consent was obtained, participants' detailed demographic information was recorded. First void urine was collected from all women. According to Nugent's criteria, BV was indeed diagnosed. All of the qPCRs performed on the FVU and vaginal swabs for the selected vaginal bacteria (Atopobium vaginae, Prevotella spp., Gardnerella vaginalis, bacterial vaginosis-associated bacterium 2, Eggerthella-like bacterium, "Leptotrichia amnionii," Megasphaera type 1) had an area under the receiver operating characteristic (ROC) curve of >85%, All data was examined using SPSS 24.0.
Results: In current study, mean age of the women was 28.11±15.68 years and had mean BMI 24.9±7.49 kg/m^2. Majority of the females were nulliparous 67 (67%). Most of the females had no genital hair remove among 60 (60%). Among 100 females, 49 (49%) females had bacterial vaginosis, 40 (40%) had normal vaginal flora and 11 (11%) had intermediate flora. Nugent scoring was used to determine the sensitivity and specificity of seven assays for BV-associated bacteria in urine (A. vaginae, Prevotella spp., G. vaginalis, BVAB 2, Eggerthella-like bacterium, L. amnionii, and Megasphaera type 1). By qualitative detection, the assays were 80%-100% sensitive but only 8%-80% specific (presence or absence). The area under the curve (AUC) was > or = 91% for all seven bacterial species, indicating high diagnostic accuracy.
Conclusion: Our research of ROC curves led us to the conclusion that FVU can be used to accurately diagnose BV. Combining qPCR findings for two bacteria, like Megasphaera type 1 and Prevotella spp., can improve sensitivity or specificity, respectively.
Keywords: First Void Urine, BV, PCR, Nugnet scoring. 7-Assays

INTRODUCTION

Alterations to the vaginal microbiota are the root cause of the clinical illness known as bacterial vaginosis (BV).[1] Communities of facultative and anaerobic bacteria replace the normally dominating lactobacilli species. Gardnerella vaginalis, Atopobium vaginae, Prevotella, Mycoplasma, and a plethora of other bacteria are only a few examples. [2,3]

There are a number of variables, including socioeconomic status, diagnostic criteria, gestational age, vitamin D insufficiency, and smoking, that have been linked to the increased incidence of BV in women.

[4] There is a very high rate of BV in South Africa, making it Africa's most afflicted country. In a population of pregnant women in the Gauteng province, the prevalence of BV was observed to be 17.6%. [5] Recent work by our team found that 49.4% of Durban antenatal women were infected with BV. [6]

Men who are in relationships with women who have bacterial vaginosis (BV) have been linked to an increased incidence of nongonococcal urethritis (NGU) [7], albeit this is an area that has received little research attention. As the most prevalent vaginal infection in women, BV is present in 10-20% of Scandinavian women [8], with symptoms include abnormal vaginal discharge, foul odour, itching, and burning, while many women either have no symptoms or have become acclimated to the condition [9]. A increased risk of premature delivery, HIV transmission, other sexually transmitted diseases, and pelvic inflammatory illness has been linked to it [10,11]. Gardnerella vaginalis, Atopobium vaginae, Prevotella spp., Eggerthella-like uncultured bacteria, Megasphaera-like type 1, Sneathia spp., and Bacterial Vaginosis Associated Bacterium-2 (BVAB-2) are the most common anaerobic bacteria found in the vagina with BV [12].

Recently published [13,14] studies have employed polymerase chain reaction (PCR) to detect BV-associated bacteria in vaginal swabs, making this method the gold standard for BV diagnosis. It is possible that first-void urine (FVU) is the sole sample used to diagnose BV in certain research since vaginal swabs are not obtained. Urine is easily collected for BV research since it is used in the standard screening for hyperglycemia and leukocytes in pregnant women. Since the Nugent score is not as sensitive or specific as a panel of BV-associated bacteria in predicting BV in vaginal swabs, we used receiver operating characteristic (ROC) curve analysis to investigate whether BV may be identified from FVU by PCR[15]. Through the examination of samples with both normal and BV floras, the Nugent score is used to create a threshold/cutoff for optimum diagnosis of BV by quantitative PCR (qPCR). Findings that factor in cutoffs are called "quantitative detection," while presence/absence results are called "qualitative detection."

MATERIAL AND METHODS

This Cross-sectional study was conducted at Hameed Latif Hospital Lahore, during from Sep, 2021 to Feb, 2022 and comprised of 100 women. Once informed written consent was obtained, participants' detailed demographic information was recorded. Females had severe medical illness, <20 years of age and those did not provide any written consent were not included.

Women had age 20-65 years. The Gram staining and Nugent scoring of vaginal smears served as the diagnostic method. There was a general consensus that flora with a Nugent score of 0–3 represented typical flora (Nugent grade I), that 4–6 represented an intermediate range, and that 7–10 represented BV (Nugent grade III). One researcher (R.D.) who was blinded to the clinical and laboratory findings rated all of the slides.

DNA was extracted from cultures of Atopobium vaginae (CCUG 38953T), "Leptotrichia amnionii" (DSM 16630) (not a validly described species but referred to by this name in accordance with the DSMZ designation), Gardnerella vaginalis (ATCC 3717T), and Prevotella bivia (CCUG 38953T) to use as positive controls for the PCRs (clinical isolate identified by biochemical properties and matrix-assisted laser desorption ionization–time of flight [MALDI-TOF] mass spectrometry). The 16S rRNA gene PCR result from a positive clinical sample was

utilised as a positive control after gel purification and DNA sequencing for the verification of the three uncultured bacteria (BVAB 2, Megasphaera type 1, and Eggerthella-like bacterium). A standard curve for quantitative PCR was constructed by diluting 1 g/ml of calf thymus DNA by 10-fold, from 107 geq/l to 1 geq/l, in TE buffer (D-8661; Sigma-Aldrich).

Bacterial load providing the best threshold for detecting BV in FVU was determined using ROC curve analysis, with sensitivity and specificity weighted equally, and normal flora and BV as identified by Nugent score serving as the gold standard. To determine whether or whether any of the bacteria tested in the FVU sample was substantially linked with BV, we used a Fisher's exact test (univariate analysis) using odds ratios (OR) and confidence intervals (CI) (before and after applying ROC curve analysis).

RESULTS

Mean age of the women was 28.11±15.68 years and had mean BMI 24.9±7.49 kg/m^2. Majority of the females were nulliparous 67 (67%). Most of the females had no genital hair remove among 60 (60%).(table 1)

Table-1: Characteristics of included women

Variables	Frequency	Percentage
Mean age (years)	28.11±15.68	
Mean BMI (kg/m^2)	24.9±7.49	
Nulliparous		
Yes	67	67
No	33	33
Genital hair removed		
Yes	40	40
No	60	60

Among 100 females, 49 (49%) females had bacterial vaginosis, 40 (40%) had normal vaginal flora and 11 (11%) had intermediate flora.(fig 1)

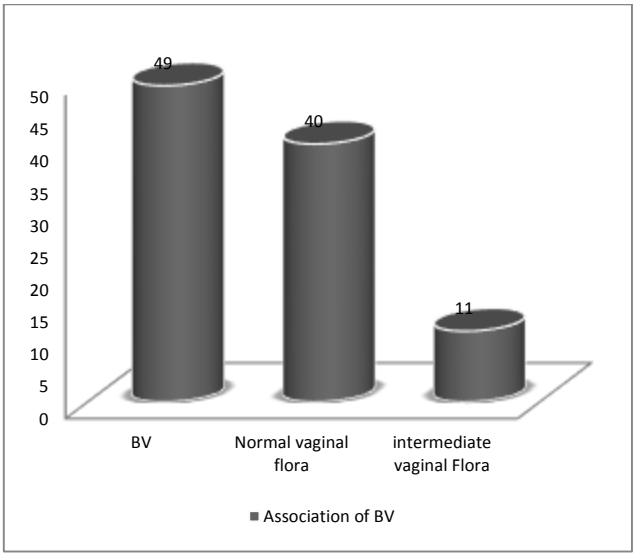

Figure-1: Frequency of BV by Nugent's Criteria

Nugent scoring was used to determine the sensitivity and specificity of seven assays for BV-associated bacteria in urine (A. vaginae, Prevotella spp., G. vaginalis, BVAB 2, Eggerthella-like bacterium, L. amnionii, and Megasphaera type 1). By qualitative detection, the assays were 80%-100% sensitive but only 8%-80% specific (presence or absence). The area under the curve (AUC) was > or = 91% for all seven bacterial species, indicating high diagnostic accuracy.(table 2)

Table-2: Precision and sensitivity prior to (qualitative detection)

Variables	Specificity	Sensitivity
Atopobium vaginae	95	15–42
Leptotrichia amnionii	88	55–75
BVAB 2	92	70–90
Megasphaera type 1	93	35-62
Eggerthella-like bacterium	90	65-82
Gardnerella vaginalis	98	4-19
Prevotella spp.	99	6-23

G. vaginalis had an AUC of 98%, A. vaginae of 97%, Prevotella spp. of 96%, BVAB 2 of 95%, an Eggerthella-like bacteria of 94%, Megasphaera type 1 of 91%, and L. amnionii of 88%. G. vaginalis showed the best sensitivity (92%) for quantitative detection, followed by A. vaginae (91%) and Prevotella spp. (90%) while the Eggerthella-like bacteria (100%), Megasphaera type 1 (99%), and A. vaginae (99%) had the highest specificities. There was no statistically significant difference between PCRs performed on swabs and those performed on FVU samples in terms of sensitivity or specificity.(table 3)

Table-3: Precision and sensitivity prior to (quantitative detection)

Variables	Specificity	Sensitivity
Atopobium vaginae	97	90-100
Leptotrichia amnionii	85	80-95
BVAB 2	96	70–90
Megasphaera type 1	95	91-100
Eggerthella-like bacterium	92	93-100
Gardnerella vaginalis	100	86-100
Prevotella spp.	100	84-96

DISCUSSION

Penile skin, sperm, and urine samples have all been shown to contain BV-associated organisms [16]. Liu et al. [17] found that the BV-like microbiota in the coronal sulcus skin specimens were a close match to the vaginal microbiota of the women they studied, but this does not prove that males should be given antibiotics to avoid recurrence of BV in their female partners. It's not quite apparent what part anaerobic bacteria play in causing urethritis in males. Only Megasphaera-like type 1 was significantly linked with IU (ORadj = 4.6), and it was discovered more often and at greater loads in IU than in controls (although this difference did not achieve statistical significance). Megasphaera-like type 1 is an unclassified vaginal bacteria that was found lately. It may be killed by metronidazole and clindamycin in the lab [18].

In our study 100 women with ages 20-65 years were presented. Mean age of the women was 28.11±15.68 years and had mean BMI 24.9±7.49 kg/m^2. Majority of the females were nulliparous 67 (67%). Most of the females had no genital hair remove among 60 (60%). These findings were comparable to the previous studies.[19,20] Recently, the use of FISH to examine G. vaginalis biofilm in urine-harvested cells was described [21]. However, a thorough validation of this method was not provided. Seven BV-associated bacteria (A. vaginae, Prevotella spp., G. vaginalis, BVAB 2, an Eggerthella-like bacterium, L. amnionii, and Megasphaera type 1) were chosen for this investigation due to their documented strong discriminatory power in diagnosis of BV from swabs [22], with areas under ROC curves >85%. As multiple species of the Prevotella genus are present in the vagina, an assay for Prevotella sp. was selected to reduce the number of tests required to cover this species. The assay's high sensitivity suggested that this strategy was working.

Among 100 females, 49 (49%) females had bacterial vaginosis, 40 (40%) had normal vaginal flora and 11 (11%) had intermediate flora.[23] Further, as a strong correlation was found between urine and swabs (R = 0.63, p 0.0001), the results of the current investigation suggested that urine is an adequate sample for detection and quantification of G. vaginalis. Additional research confirms these results. Swidsinski et al.[24] found that G. vaginalis was present in the urine of German pregnant women who had just finished their first void. A more recent study by Datcu et al.[19]

showed that it was possible to identify G. vaginalis in the urine of Greenland women from the general community. Both qualitative and quantitative detection of all seven bacterial species in urine were strongly correlated with BV in univariate analysis, however in a multivariate logistic regression model, only Megasphaera type 1 and Prevotella spp. were significantly correlated with BV. In contrast, the results of a multivariate analysis of swabs from the same women showed that A. vaginae and Prevotella spp. were related with BV. There has been no explanation for this disparity.

Women with BV had a higher median bacterial load in their urine than other women, with Prevotella spp. being the most common of the species discovered in FVU. Samples with moderate scores (Nugent grade II) were omitted from the ROC curve analysis used to identify the best cutoff amount. Whether or if the ability to categorise intermediate flora into two groups will help researchers better understand the links between diseases in the future is uncertain. For all seven species, the median DNA load in swabs was greater than the equivalent load in FVU specimens, however there was a linear relationship between the vaginal and urine bacterial loads in all tests. Therefore, the DNA loads found in the FVU samples likely reflect vaginal secretions washed away by the urine rather than a urinary tract infection (UTI), while the latter hypothesis should be explored in future investigations on midstream pee. Considering that DNA loads for all seven species were more than 108 copies per ml of FVU in certain samples, colonisation of the bladder epithelium should not be ruled out as a possible outcome. The endometrium and fallopian tubes of 25% of women with BV, according to a recent study by Swidsinsky et al.[25].

CONCLUSION

Our research of ROC curves led us to the conclusion that FVU can be used to accurately diagnose BV. Combining qPCR findings for two bacteria, like Megasphaera type 1 and Prevotella spp., can improve sensitivity or specificity, respectively.

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