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Original Article

Prevalence and associated factors of bacterial vaginosis among pregnant women in Hue, Vietnam

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Abstract

Introduction: Bacterial vaginosis (BV) is the most frequent vaginal infection affecting women of childbearing age worldwide. It is associated with significant adverse healthcare outcomes, especially during pregnancy. Although screening for BV could reduce potential pregnancy-related obstetric complications, there is no routine screening of pregnant women for BV in Vietnam. We aimed to identify the prevalence of BV among pregnant women and the associated factors in two tertiary hospitals in Hue, Vietnam.

Methodology: This cross-sectional descriptive study included 885 pregnant women in third trimester, who received routine antenatal care in the Hue Central Hospital and Hue University Hospital of Medicine and Pharmacy, Hue city, Thua Thien Hue province, Vietnam. Gram-stained vaginal smears were used for calculating the Nugent score and recording the fungal elements.

Results: In total, 435 (49.1%) women had a normal BV score, 352 (39.8%) had intermediate vaginal microbiota, and 98 (11.1%) had BV. Among the 98 women with BV, 71 (72.4%) also had fungal infection. There was a significant association of BV with discharge ($p = 0.004$) and abnormal cervix ($p = 0.014$). BV was significantly more frequent among the women who reported previous abortion or miscarriage ($p = 0.007$).

Conclusions: About a tenth of women in Thua Thien Hue province have BV in the third trimester of pregnancy being associated with previous adverse outcome. Discharge with fishy odour is still a characteristic feature among subtle clinical presentations of BV. Better awareness about this disease and routine test-and-treat management during pregnancy may improve pregnancy outcome.

Key words: vaginosis; pregnancy; outcome; yeast.

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Introduction

The diverse and dynamic vaginal microbial community consists of a variety of bacteria, that, in healthy women, contain numerous lactobacilli. The microbial community fluctuates throughout women's lives, depending on age, estrogen levels, sexual behaviors, and environment [1,2]. Women's health and the health of the fetuses (including reproductive function) are significantly influenced by the vaginal microbiota [3].

In 1955, Gardner and Dukes published the first description of bacterial vaginosis (BV), including the distinguishing clinical symptoms and characteristics of vaginal discharge associated with the condition [4]. BV is a dysbiosis of the vaginal microbiota characterized by a shift from dominant lactobacilli to a polymicrobial community [5]. The polymicrobial community consists

of bacteria such as *Gardnerella vaginalis*; anaerobic bacteria such as *Prevotella* spp., *Peptostreptococcus* spp., *Mobiluncus* spp., and *Sneathia* spp.; mycoplasmas (*Mycoplasma hominis*, *Ureaplasma urealyticum*); and several other bacteria which replace the lactobacillus populations that are typically prominent in healthy women [6]. BV patients often experience uncomfortable symptoms like grey watery vaginal discharge and/or foul "fishy" odor [7]. Amsels' criteria are frequently used for diagnosing BV in clinical practice. Amsel's criteria include nature of discharge, increased vaginal pH (> 4.5), amine odor after the addition of 10% potassium hydroxide, and detection of clue cells (vaginal epithelial cells heavily coated with bacteria) in vaginal secretion [8]. Laboratory confirmation is mostly done using Gram-stained slides and Nugent scoring—which are considered reference standard methods [9,10]. Treatment options for BV include oral or topical metronidazole or clindamycin. It is important to distinguish between BV and aerobic vaginitis (the latter is characterized by yellowish discharge, rotten odor, vaginal redness and dyspareunia) because they require different treatment regimens.

BV is the most frequent vaginal infection affecting women of childbearing age worldwide [8, 11–13], especially those aged between 15 and 44 years. Nugent-diagnosed BV was identified in 23–29% women of reproductive age globally, in seven areas of the world [14,15].

BV is associated with significant adverse healthcare outcomes, including increased susceptibility to sexually transmitted infections (such as HIV, gonorrhea, trichomoniasis, and genital herpes), pelvic inflammatory diseases, urinary tract infections; and an increased risk of abnormal pregnancy outcome [16–19]. The latter includes increased risk of miscarriage, preterm labor, chorioamnionitis, neonatal infections, and postpartum complications including endometritis and wound infections [20–22]. These conditions are associated with a higher risk of additional health consequences.

Although screening for BV could reduce potential pregnancy-related complications, there is no routine BV screening for pregnant women in Vietnam. We carried out this study to reveal the prevalence of BV among pregnant women and the associated risk factors in two tertiary hospitals in Hue, Vietnam.

Methodology

Study subjects

A total of 885 participants were recruited for this cross-sectional descriptive study between July 2018 and January 2019, from among pregnant women (≥ 28 weeks) who received routine antenatal care at the Hue Central Hospital (n = 613) and Hue University of Medicine and Pharmacy (Hue UMP) Hospital (n = 272), Hue city, Vietnam. All the women had viable, normal morphological fetuses. Exclusion criteria included previously diagnosed rupture of membranes, antepartum hemorrhage, vaginal douching before or during vaginal specimen collection, and treatment for reproductive tract infections or use of antibiotics for any other reason within the preceding week.

The sample size was calculated according to a previous study from the Hue UMP Hospital that reported that 42.9% of pregnant women in the third trimester possess bacteria that might lead to infections of the vaginal tract [23]. We considered a confidence interval of 95% and a relative deviation of 0.16, and estimated that our study required a sample size of at least 200 study subjects in each hospital.

The Ethics Committee of Hue University of Medicine and Pharmacy approved this research (No. H2018/162 dated 24 May 2018). Participation in the research was entirely optional. All research participants gave written consent.

Clinical investigations and specimen collection

Pregnant women in their third trimester of pregnancy (≥ 28 weeks) who were eligible for treatment and/or labor at Hue tertiary hospitals were asked for general information, undergoing a clinical examination and sampling. The demographic information included age, education, profession, marital status, and history of reproductive health (genital tract infections, abortions, and miscarriages).

Clinical data from the previous month were recorded, including discharge, itching, and abnormal vaginal bleeding in their medical recordings. Gynecological examination included examination of the condition of vulva, vagina, and cervix; as well as the nature and amount of discharge.

Vaginal fluid was collected from the posterior vaginal fornix. A sterile cotton swab was used to collect sample, which then delivered to the Department of Microbiology at the Hue UMP Hospital within 2 hours or stored at 4 °C within 12 hours before sending to the lab. The swab was used to produce a Gram-stained slide smear.

Table 1. Nugent scoring system for Gram-stained vaginal smears.

Score *	Gram-positive rods (*Lactobacillus* morphotypes)	Small Gram-variable rods (*Gardnerella*/*Bacteroides* morphotypes)	Curved Gram-variable rods (*Mobiluncus* morphotypes)
0	4+	0	0
1	3+	1+	1+ or 2+
2	2+	2+	3+ or 4+
3	1+	3+	
4	0	4+	

* Morphotypes are counted as the average number seen per oil immersion field. Each morphotype was quantitated from 0 to 4+ (0: no morphotypes; 1+: less than 1 morphotype; 2+: 1 to 4 morphotypes; 3+: 5 to 30 morphotypes; 4+: 30 or more morphotypes). Total score was sum of three sub-scores. Total score between 0 and 3 corresponded to the normal vaginal microbiota: values between 4 and 6 indicated an intermediate vaginal microbiota: and values between 7 and 10 indicated bacterial vaginosis (BV). Adapted from [9].

Laboratory methods

BV was recognized according to the Nugent score that was calculated by examining the Gram-stained slides under oil immersion microscopy (1000x magnifications). Gram-positive rods (*Lactobacillus* spp. morphotypes), small Gram-variable rods (*Gardnerella vaginalis*/*Bacterides* spp. morphotypes) and curved Gram-variable rods (*Mobiluncus* spp. morphotypes) were counted. Regarding the quantity of morphotypes in each oil immersion field, each morphotype was rated from 0 to 4+ (0, no morphotypes; 1+, less than 1 morphotype; 2+, 1 to 4 morphotypes; 3+, 5 to 30 morphotypes; 4+, 30 or more morphotypes). In the case of *Lactobacillus* spp. morphotypes, this scale was in the reverse direction, with no morphotypes equaling 4. Values between 0 and 3 corresponded to the normal vaginal microbiota, values between 4 and 6 indicated intermediate vaginal microbiota, and values between 7 and 10 were regarded as diagnostic for BV (Table 1) [9]. Vaginal fungal infection was also detected by the same Gram-stained slides based on the presence of yeast blastospores and/or pseudohyphae.

Statistical methods

MS Excel 2016 was used for data recording. The data were processed and analyzed using SPSS 20.0 software. The Chi-squared test or Fisher exact test for categorical variables were applied to analyze the associations between BV and clinical factors. $p < 0.05$ was considered statistically significant.

Results

Sociodemographic and clinical characteristics of the study subjects

A total of 885 women with an average age of 28.2 years participated in this research. The majority of the women (62.1%) were between the ages of 25 and 34 years, and 94.6% had secondary educational level or

Table 2. Background characteristics of the study group.

	BV (n, %)	Non-BV (n, %)	*p* value
Age (years)			0.740
< 18	0 (0%)	11 (1.3%)	
18–24	7 (18.9%)	204 (24.1%)	
25–34	24 (64.9%)	525 (61.9%)	
≥ 35	6 (16.2%)	108 (12.7%)	
Profession			0.201
Officer	9 (24.3%)	230 (7.1%)	
Worker	5 (13.5%)	230 (7.1%)	
Housewife	12 (32.4%)	202 (23.8%)	
Other	11 (29.7%)	186 (21.9%)	
Education level			0.969
Illiteracy	0	0	
Primary school	2 (5.7%)	45 (5.4%)	
Secondary or high school	24 (68.6)	592 (70.6)	
College or above	9 (25.7%)	202 (24.1%)	
Marital status			0.807
Married	37 (100%)	842 (99.4%)	
Unmarried	0 (0%)	5 (0.6%)	
Sampling time (week of pregnancy)			0.696
< 37	3 (8.1%)	55 (6.5%)	
≥ 37	34 (91.9%)	793 (93.5%)	
History of lower genital tract infections			0.877
Yes	3 (8.1%)	75 (8.8%)	
No	34 (91.9%)	773 (91.2%)	
History of abortion or miscarriage			0.007*
Yes	2 (5.4)	7 (0.8%)	
No	35 (94.6%)	841 (99.2%)	

*Significant value.

above. Nearly half of the women (48.8%) were public servants, and 99.4% of the women were married. History of lower genital tract infections were reported in 8.8% of pregnant women. BV was significantly more frequent among the women who reported previous abortion or miscarriage *(p* = 0.007) (Table 2).

During the previous month, discharge was noted in 7.1% of women, most commonly white cottage cheese-like discharge (in 5.2% of women). Vaginal itching was noted in 2.1% and abnormal bleeding in 0.2% of women during the previous month. Based on gynecologic examination, discharge was recorded in 15.3% of women. Abnormalities in vagina and cervix were noted in 7.6% and 6.2% women respectively, and was mostly described as inflammation (Table 3).

Prevalence and associations of bacterial vaginosis in pregnant women

According to Nugent's classification, 49.1% of women (435 cases) had a normal microbiota (Nugent score 0 to 3), 39.8% (352 cases) had an intermediate vaginal microbiota (Nugent score 4 to 6), and 11.1% (98 cases) had BV (Nugent score 7 to 10). Of the 98 women with BV, 71 (72.4%) also had fungal infection.

Majority of the women with BV had no symptoms within the previous month; however, a significant difference was observed in the discharge in women with and without BV *(p* < 0.01). There was no significant difference in other symptoms, including vaginal itching and abnormal vaginal bleeding between women with and without BV (*p* > 0.05). Based on gynecologic

Table 3. Clinical profiles of pregnant women and their association with BV.

Clinical characteristic			Prevalence in group (n = 885)		Prevalence (n) in the women with different microbiota conditions			*p* value
			n	%	Normal (n = 435)	Intermediate (n = 352)	BV (n = 98)	
Expression within the last 1 month								
Discharge	**Yes**		63	7.1	27	21	15	**0.004**
	No				408	331	83	
	1. Clear		5	0.6	4	1	0	
	2. Yellow, green, bubble		8	0.9	4	3	1	
	3. White, cottage cheese like		46	5.2	19	16	11	**0.011**
	4. Pus-like		2	0.2	0	1	1	
	5. With blood		1	0.1	0	0	1	
	6. Other		1	0.1	0	0	1	
Vaginal itching	**Yes**		19	2.1	8	6	5	0.114
	No				427	346	93	
Abnormal vaginal bleeding	**Yes**		2	0.2	0	1	1	0.1
	No				435	351	97	
Expression during gynecologic examination								
Vulva	**Abnormal**		2	0.2	1	0	1	0.209
	Normal				434	352	97	
	1. Itching		1	0.1	0	0	1	1
	2. Inflammation		1	0.1	1	0	0	
Discharge	**Abnormal**		135	15.3	77	37	21	**0.004**
	Normal				358	315	77	
	1. Clear		5	0.6	1	3	1	
	2. Yellow, green, bubble		17	1.9	8	6	3	
	3. White, cottage cheese like		106	12.0	64	27	15	**0.004**
	4. Pus-like		2	0.2	0	1	1	
	5. Other		5	0.6	4	0	1	
Vagina	**Abnormal**		67	7.6	35	20	12	0.085
	Normal				400	332	86	
	1. Inflammation	Yes	65	7.3	35	18	12	0.04
		No			400	334	86	
	2. Ulcer	Yes	0	0	0	0	0	
		No			435	352	98	
	3. Wart	Yes	1	0.1	0	1	0	0.508
		No			435	351	98	
Cervix	**Abnormal**		55	6.2	27	16	12	**0.02**
	Normal				408	336	86	
	1. Inflammation	Yes	54	6.2	27	15	12	**0.014**
		No			408	337	86	
	2. Ulcer	Yes	0	0	0	0	0	
		No			435	352	98	
	3. Wart	Yes	1	0.1	1	0	0	1
		No			434	352	98	
	4. Bleed	Yes	3	0.3	2	1	0	1
		No			433	351	98	

BV: bacterial vaginosis.

examination, the most remarkable differences between the women with and without BV were in the case of discharge (p = 0.004) and cervical inflammation (p = 0.014). No significant differences were noted in the case of other vaginal and cervical parameters between the women with and without BV ($p > 0.05$) (Table 3).

Discussion

The prevalence of bacterial vaginosis was 11.1% among pregnant women in their third trimester in Thua Thien Hue Province, Vietnam. Based on gynecologic examination, there were significant differences in discharge and its properties, as well as abnormal cervical appearance among women with and without BV. Current BV was significantly associated with previous abortion and miscarriage.

Prevalence of BV during the third trimester of pregnancy

The vaginal microbiota fluctuates periodically along with environmental changes, making it a dynamic population rather than a static one. Pregnancy is as an endogenous factor that may cause the fluctuations in vaginal microbiota because of changes in immunity and hormonal levels during pregnancy [24,25]. There is more available glycogen during physiological pregnancy, and greater estrogen levels have a favorable impact on lactobacillary activity and proliferation in addition to improving epithelial tropism [26]. Previous studies have shown increase in lactobacilli counts and decrease in BV as the pregnancy progresses [27–29]. At the same time, hormonal background at the end of pregnancy supports fungal growth in vagina [4].

In our study, the prevalence of BV among the pregnant women was not high (11.1%). Based on Nugent score, 49.1% of samples were classified as normal, 39.8% as having an intermediate vaginal microbiota, and 11.1% (98 cases) as having bacterial vaginosis. This result was different from some reports in which BV was more frequent in pregnant women with unspecified pregnancy stage [11–13] while it was similar to other studies where pregnant women in the third trimester were investigated [29,30].

BV is one of the most significant risk factors for adverse pregnancy outcomes such as premature rupture of membranes, preterm labor and delivery, intra-amniotic and neonatal infection, and postpartum endometritis [10, 31–33]. This was also observed in our study. BV is not caused by a single organism but it appears to be associated with *Gardnerella vaginalis*, *Mobiluncus* spp., *Sneathia* spp., *Mycoplasma hominis* and several other bacteria, therefore, these complications may be associated with different bacteria [26]. All these bacteria belong to normal vaginal microbiota in small amounts; therefore, their detection cannot be used for diagnosing BV. Instead, Amsel's criteria and Nugent scoring should be used. Early and adequate treatment of BV may prevent adverse pregnancy outcomes [34]. Although BV discharge has a typical fishy odor, nearly half of the women with BV may be asymptomatic or only have minor symptoms [35–37]. Even the vast majority of women (84%) who participated in the National Health and Nutrition Examination Survey (NHANES) in the United States reported no symptoms, with a BV prevalence of 29.2% among women aged 14 to 49 years (which corresponds to 21.7 million women) [12]. This creates additional difficulties in the management of pregnant women. According to recent Centers for Disease Control and Prevention (CDC) guidelines, BV treatment is recommended for all symptomatic pregnant women [10]. Treatment of asymptomatic BV among pregnant women at high risk for preterm delivery has been evaluated by multiple studies, which have reported mixed results—one study reported harm, two reported no benefit, and four demonstrated benefits. Treatment of asymptomatic BV among pregnant women at low risk for preterm delivery did not reduce adverse outcomes of pregnancy in a large multicenter randomized controlled trial [38]. Therefore, routine screening for BV among asymptomatic pregnant women for preventing preterm birth is currently not recommended [10].

Associations between BV and clinical profiles of pregnant women

Clinically, BV is frequently diagnosed using Amsel's criteria that include presence of clue cells under a microscope, vaginal pH > 4.5, and thin grey vaginal discharge with a fishy odor [8]. BV is the most common cause of vaginal discharge. Other signs and symptoms like dysuria, dyspareunia, pruritus, burning, or vaginal inflammation are not often brought on by BV alone [8, 36] but they point to mixed vaginitis (symptoms due to more than one pathogen) [39]. Because of the changes in immunity during pregnancy, the prevalence of mixed infections in pregnant women may be higher than that in non-pregnant women [30]. These different conditions need careful differential diagnostics and appropriate treatment.

In our study, three quarters of women with BV also had candidiasis based on the observation of fungal elements on Gram-stained slides, and 85.7% women with BV had white cottage cheese-like discharge which

is a typical sign of fungal infection. Candidiasis is a very common vaginal infection at the end of pregnancy that is responsible for a more prominent clinical picture than BV. White curdy discharge, pruritus, vaginal soreness and dyspareunia are the common symptoms of candidiasis. Topical azole treatment for fungal infection is recommended during pregnancy [10].

In addition, in our study, abnormal cervical appearance was associated with BV and this was mostly termed as inflammation. Previous studies have reported that in some cases, BV-related bacteria may ascend and cause inflammation in cervix and upper genital tract. In addition, BV may be a predictor of other cervicitis-causing infections [40].

This study had some limitations. The Nugent scoring system was used to assess the prevalence of the various bacterial morphotypes but it did not enable differentiation between lactobacilli species, such as *Lactobacillus crispatus* and *Lactobacillus iners*. The latter has lower protective capacity against infections than *L. crispatus*. However, Nugent scoring is still considered a gold standard for detection of BV. This simple method can be easily applied in healthcare facilities. A Gram-stained smear was used to detect fungal elements. We did not culture the yeasts; hence, we could not identify *Candida* species. In addition, data on pregnancy outcome of the study group were not available. However, this study included a large number of participants, and therefore the results contribute valuable information on the prevalence of BV among pregnant women in Hue city, Thua Thien Hue Province, Vietnam.

Conclusions

About a tenth of women in Thua Thien Hue province, Vietnam had BV in the third trimester of pregnancy and this was associated with previous adverse outcome. Although BV may present with a very subtle clinical presentation, its discharge with fishy odour is still a characteristic feature of this pathogen. Better awareness about this disease and routine test-and-treat management during pregnancy may improve pregnancy outcome.

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