



Original Research Article

Screening of Bacterial Vaginosis in Pregnant Women of Bundelkhand Region

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Abstract

Bacterial Vaginosis (BV) is one of the most common vaginal infection occurring in the women in the reproductive age group (pregnant and non pregnant), where the normal microflora of vagina i.e. Lactobacillus is replaced by an overgrowth of pathogenic bacteria like Gardnerella vaginalis, Mobilincus and Bacteroides species. Early screening of bacterial vaginosis in pregnant women shall prevent adverse outcomes. A total of 107 pregnant women (symptomatic) vaginal samples were collected visiting Obstetrics and Gynecology Department of Maharani Laxmi Bai Medical College, Jhansi. Three high vaginal swabs taken for each symptomatic patient from the upper part of posterior fornix. Morpho-texture of vaginal discharge and pH of the discharge was measured. The clinical criteria i.e. Amsel Criteria was used in the study for BV. Further gram staining was done to quantify bacterial morphotypes, to identify clue cells and scored for the diagnosis of bacterial vaginosis following Nugent's scoring system. Out of 107 symptomatic samples, 52% cases were positive for bacterial vaginosis as per Amsel's criteria. A significantly high incidence was shown in first trimester of pregnancy (0-3months) (33%) followed by cases of third trimester (7-9 months) (21%) and in second trimester (4-6 months) it lies to 17%. Among 107 samples, the prevalence was highest in pregnant women with first pregnancy (21%), followed by second pregnancy (15%) and with third or more pregnancy (13%). The study showed the prevalence of bacterial vaginosis among pregnant women of Bundelkhand region, including all three trimesters and various pregnancy scores. Pregnant women should be screened and treated for bacterial vaginosis to reduce risk of recurrence and adverse pregnancy complications.

Keywords: Bacterial Vaginosis, vaginal swab, Clue cells, Nugent scoring system.

Introduction

Bacterial Vaginosis (BV) is a condition of alteration in the normal microflora of vagina i.e. hydrogen-peroxide producing Lactobacillus is replaced by an overgrowth of pathogenic bacteria like Gardnerella vaginalis, Mobilincus and Bacteroides species. Many factors are responsible

for changes in the vaginal microflora including concomitant infection, sexual activity, douching, and number of sexual partners, smoking, menstruation and contraceptive methods^[1].

It is one of the most prevalent vaginitis and is responsible for approximately one third of all cases of vulvovaginitis in women of reproductive

age group. The markable symptoms are excessive discharge and odor. The diagnosis of this condition is likely when a patient complains of a malodorous, non irritating discharge, and on examination reveals homogeneous grey white secretions, but it is encountered that more than one half of patients with demonstrable signs have no symptoms.

A high concentration of *G.vaginalis* is often associated with the presence of BV^[2]. It is a very common infection in women, and there is a lack of understanding regarding the triggers and factors for the onset and resolution of it^[3]. It can lead to serious adverse pregnancy complications like preterm labor pains and preterm delivery, premature rupture of membranes (PROM), spontaneous abortion, and postpartum endometritis^[4]. Early screening of bacterial vaginosis in pregnant women shall prevent such adverse outcomes^[5].

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The cross sectional descriptive study was done to see the prevalence of BV in pregnant women attending Obstetrics and Gynecology Department of Maharani Laxmi Bai Medical College, Jhansi including parameters of trimesters of pregnancy and scores of pregnancy.

Materials and Methods

Total of 107 pregnant women, vaginal samples were collected visiting OPD of Obstetrics and Gynecology Department of Maharani Laxmi Bai Medical College, Jhansi for a period of 4 months (26 October 2018 to 26 February 2019) with

complaints of one or more symptoms suggesting lower genital tract infection like malodorous discharge from vagina, itching, burning micturition and dysuria. An informed and written consent was taken from each of participants. A self-designed detailed questionnaire of the patient was filled that included general patient health history, number of pregnancy, educational level, age, ethnic group etc for covering the variables associated with the study.

Approval for conducting the study was taken from the Ethical Committee of the Medical Institute. Non-pregnant women, non symptomatic pregnant women, immunocompromised patients having underlying debilitating disease or malignancies or women on antibiotics in the preceding four weeks were excluded from the study.

Three high vaginal swabs taken for each symptomatic patient from the upper part of posterior fornix and lateral vaginal walls using sterile cotton-tipped swab. Morpho-texture of vaginal discharge and pH of the discharge was measured. The vaginal swabs were processed in the microbiology laboratory by wet mount examination, Gram's staining, Whiff's test and pH determination. With one swab pH of the discharge was measured, by rubbing swab against a narrow range pH paper. Whiff's test (Amine test) was performed by adding two drops of 10% potassium hydroxide on the second swab. Development of an amine fishy odor was considered as a positive test. The third swab was used for wet mount and Gram's staining. For wet mount examination, the secretion from the swab was smeared on a clean glass slide and was later covered with the cover slip. The slide was observed for the presence of 'Clue cell'. Clue cells are examined by their edges. Normal squamous cells have clear linear edges whereas a clue cell has granular, cloudy and rough edge.

For Gram staining the swab is again smeared on another clean slide. Its then air dried, fixed with the gentle heat and the stained by Gram's staining method. After air drying the smear it will be examined under oil immersion objective for short

Gram negative or Gram variable bacilli (*Gardnerella vaginalis*), Curved Gram negative bacilli (*Mobiluncus*), epithelial cells with heavy coating of Gram negative bacilli on the periphery

(clue cells) and thick Gram positive bacilli (Lactobacilli). The smear will be graded and interpreted based on Nugent’s score.

Table 1: Nugent’s scoring of vaginal swabs for diagnosis of bacterial vaginosis.

Morphotype	Number of organisms per oil immersion field				
	None	<1	1-4	5-30	>30
Lactobacillus species	4	3	2	1	0
Gardnerella & anaerobic Gram negative bacilli	0	1	2	3	4
Curved Gram negative bacilli (<i>Mobilincus</i> species)	0	1	1	2	2

Table 2: Number based on average of 10 fields

Interpretation of Nugent’s score		
Nugent’s score	And	Interpretation
0-3	No clue cells	Normal vaginal flora
4-6	No clue cells	Intermediate or Not consistent with Bacterial vaginosis
4-6	Clue cell present	Indicative of bacterial vaginosis
≥ 7	Clue cell present or absent	Indicative of bacterial vaginosis

The clinical criteria (Amsel Criteria) used in the study for BV patients were:

- Increased homogenous grayish-white vaginal discharge
- Increased vaginal pH>4.5
- A fishy smell on addition of 10% KOH to vaginal fluid (Whiff test)
- Presence of clue cells on a wet mount preparation.

If more than three criteria were detected, the patient was considered to have bacterial vaginosis or to be ‘positive’ [6, 7]. Further gram staining was done to quantify bacterial morphotypes, to identify clue cells and scored for the diagnosis of bacterial vaginosis following Nugent’s scoring system. The Nugent scoring system is built on standardized Gram-stain interpretation-based criteria where vaginal swab smears are graded on a 10-point scale according to presence or absence of *Lactobacillus* morphotypes, Gram-variable or

Gram-negative rods, and curved Gram-negative rods^[10]. The Nugent score was designed to evaluate the alterations in vaginal microbiota from the healthy to the BV state, and is considered the gold standard in BV diagnosis^[8,9,10]. A score of 0–3 is considered normal, 4–6 intermediate, 7–10 positive for BV^[10].

Results

Out of total 107 pregnant women screened 68 (63.55%) were out-patients and 39 (36.44%) were in-patients. On the basis of clinical history details the patients were categorized for White discharge per vagina, any other type of discharge, itching, burning micturition and history of UTI. WDPV was the most complaint and reported with 68 (63.55%) patients. Table 3 shows the history details of the symptomatic pregnant women included in the study.

Table 3: History details of participants

History	Number Present (%)	Number Absent (%)
WDPV [#]	68(63.55)	39(36.44)
Itching	47(43.92)	60(56.07)
Burning Micturition	56(52.33)	51(47.66)
Other discharge	44(41.12)	63(58.87)
Recent UTI [#]	23(21.49)	84(78.50)

[#]WDPV- White discharge per vagina; [#]UTI Urinary tract infection

The study included two parameters trimester of pregnancy and score of pregnancy to see the prevalence of bacterial vaginosis in the Bundelkhand region on the basis of the above stated parameters. Out of 107 participants, 46 were in first trimester, 30 were in second trimester and rests 31 were in their third trimesters of pregnancy. A significantly high incidence was shown in first trimester of pregnancy (0-3months) (33%) followed by cases of third trimester (7-9 months) (21%) and in second trimester (4-6

months) it lies to 17%. The prevalence of BV in pregnant women with respect to their trimesters of pregnancy has been illustrated in Table 4. On account of second parameter, regarding score of pregnancy, among 107 samples, the prevalence was highest in pregnant women with first pregnancy (21%), followed by second pregnancy (15%) and with third or more pregnancy (13%). Table 5 shows the prevalence of BV along with score of pregnancy.

Table 4: Prevalence of bacterial vaginosis among pregnant women with different Trimesters of pregnancy

Trimesters of pregnancy	Number of participants (%)	Infection of BV	
		Positive cases (%)	Negative cases (%)
First trimester (0-3 months)	46(43)	15 (33)	31 (67)
Second trimester (4-6 months)	30(28.03)	05 (17)	25 (83)
Third trimester (7-9 months)	31(28.97)	06 (21)	25 (79)

Table 5: Association between bacterial vaginosis with different number of pregnancy

Scores of pregnancy	Number of participants (%)	Infection of BV	
		Positive cases (%)	Negative cases (%)
First pregnancy	36(43)	15 (33)	31 (67)
Second pregnancy	30(28.03)	05 (17)	25 (83)
Third pregnancy	48(28.97)	06 (21)	25 (79)

Discussion

The vaginal microflora is predominantly composed of Lactobacillus. Lactobacillus plays important role to maintain vaginal ecosystem by preventing overgrowth of potentially pathogenic species. Decrease in lactobacillus may disturb the normal microbiota status of vagina. Bacterial vaginosis occurs when this normal flora is disrupted and further replaced by overgrowth of opportunistic pathogens like Gardnerella vaginalis, Molbiluncus species, Bacteroides, Prevotella species etc^[9,10,11]. It is associated with various adverse health outcomes like preterm birth and acquisition of sexually transmitted infections^[11]. In case of pregnant women it may lead to serious complications such as preterm labor pains and preterm delivery, premature rupture of membranes (PROM), chorioamnionitis, spontaneous abortion, and postpartum

endometritis. Various other risk factors like smoking, sexual activity, poor hygiene and vaginal douching have been associated with BV in pregnant women^[12,13].

In this study, the pregnant women selected for the study were symptomatic having one or more of the complaints like vulvar irritation, itching, burning micturition, white discharge or any other kind of discharge per vagina. Most of the participants had complaint of WDPV, followed by problem of burning micturition and vaginal itching (table 3). The study has revealed that the rate of prevalence of BV was more during first trimester (33%) followed by third trimester (21%) and then by second trimester (17%). The pregnant women whose vaginal samples has been taken in first trimester had significantly shown high prevalence, this might be due to the fact that with each trimester ahead the participant might have

undergone medication or counseling by their gynecologist for their symptoms related to BV and may have improved their hygiene and sanitation practices.

The study shows the rate of infection was in the order 1st>2nd>3rd pregnancy. This might be due to the fact that with the increase in number of pregnancy the vaginal microflora becomes more consistent, with medical counseling the patient may have become more aware about the hygiene and sanitation practices and also may have undergone treatment for BV when diagnosed in previous pregnancies. S.Shrestha et al also found a significant correlation between BV and number of pregnancy.

The study revealed that if the symptomatic cases go undetected, that may cause abnormalities and complications in child birth and even on maternal health. Hence there should be strong recommendation to conduct routine BV examination of pregnant women during their prenatal visits.

Conclusion

Bacterial vaginosis is a polymicrobial infection and is much prevalent infection of reproductive age group. In pregnant women it can lead to serious critical consequences. BV has also been associated with decreased success of in vitro fertilization procedures and increased the risk of cystitis. Routine vaginal and cervical swabs should be performed on all pregnant women during their prenatal visits. Early screening of pregnant women symptomatic for BV and treatment can reduce risk of reoccurrence and adverse pregnancy complications.

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References

1. Amesl Richard, Totten A Patricia, Spiegel A Carol, Chen SC Kirk, Eschenbach David, Holmes K King; Nonspecific Vaginitis, Diagnostic Criteria and Microbial and Epidemiologic Associations. Am J med 1983 Jan; 74: 14 – 19.
2. Livengood CH (2009) Bacterial vaginosis: an overview for 2009. Rev Bras Ginicol Obstet 2: 28-37.
3. Gellar M, Nelson A. Diagnosis and treatment of recurrent and persistent vaginitis. Women's Health Gynecol. 2004; 4:137–146.
4. Thomason L Jessica, Gelbard M Sheldon, Anderson J Robert, Walt K Ann *et al.*; Statistical evaluation of diagnostic criteria for bacterial vaginosis. Am. J. Obstet and Gynaec 1990; 162:155 – 160.
5. Guise JM, Mahon SM, Aickin M, Helfand M, Peipert JF, Westhoffc. Screening for bacterial vaginosis in pregnancy. Am J Prev Med 2001 Apr; 20:62-72.
6. Srinivasan S, Fredricks DN (2008) The human vaginal bacterial biota and bacterial vaginosis. Interdiscip Perspect Infect Dis 208: 1-22
7. Amsel R, Totten PA, Splegel CA, Chen KCS, Eschenbach DA, Holmes KK (1983) Non-specific vaginitis: diagnostic criteria and microbial and epidemiologic associations. Am J Med 74:14-22
8. Martin DH (2012) The microbiota of the vagina and its influence on women's health and disease Am J Med Sci 343: 2-9
9. Filho DSC, Diniz CG, Silva VL (2010) Bacterial vaginosis: clinical, epidemiologic and microbiological features. HU Rev 36: 223-230
10. Nugent RP, Krohn MA, Hillier SL (1991) Reliability of diagnostic bacterial vaginosis is improved by a standardized method of Gram stain interpretation. J Clin Microbiol 29: 297-301

11. Purwar M, Ughade S, Bhagat B, Agarwal V, Kulkarni H (2001) Bacterial vaginosis in early pregnancy and adverse pregnancy outcome. J Obstet Gynaecol Res 27:175–181
12. Amesl Richard, Totten A Patricia, Spiegel A Carol, Chen SC Kirk, Eschenbach David, Holmes K King; Nonspecific Vaginitis, Diagnostic Criteria and Microbial and Epidemiologic Associations Am Jmed 1983 Jan; 74: 14 – 19.
13. Minkoff Howard, Grunebaum N Amos, Schwarz H Richard, Feldman Joseph, Cummings Marinella *et al.*; Risk factors for prematurity and premature rupture of membranes: A prospective study of the vaginal flora in pregnancy. Am. J. Obstet and Gynaec 1984; 150(8):965 – 971.