

## Characterizations of Bacterial Vaginosis among HIV-positive and HIV-negative Pregnant Women

Samuel M. Adelaiye\*, MB.BS, PGDCS, FWACS; S. O. Shittu, FWACS, DRH.FICS;  
A.T. Olayinka, MB.BS, FMCPPath, MPH

Federal Medical Centre, Azare, Bauchi State, Nigeria  
Ahmadu Bello University Teaching Hospital, Zaria, Nigeria

### Abstract

**Objective:** The aim of this study was to find the characteristics of bacterial vaginosis (BV) among HIV-infected and HIV-negative pregnant women at ABUTH, Zaria.

**Study design:** Case control and laboratory-based design, conducted from August 2, 2011, to December 30, 2011. BV was defined using Amsel's criteria, which uses 3 out of 4 diagnostic criteria to diagnose BV.

**Results:** Two hundred HIV-positive and 200-HIV negative women were included in the study. The mean age was 26.3 years with a range from 17 to 41 years. The prevalence rate of BV irrespective of HIV status was 33.2%. In HIV-positive pregnant women, the prevalence was 46.0%; in HIV-negative pregnant women, the prevalence was 20.5% (OR=3.30; 95% CI: 2.1-5.1,  $P<0.05$ ). Hence, HIV-positive pregnant women were significantly more likely to have BV. BV was most prevalent in the age group of 30 to 34 years, with 49% positive, and in those with parity between 1 and 4, with a prevalence of 41.5%. It is least common in primigravidae with 19%. Prevalence among HIV-positive pregnant women whose CD4 > 350cells/mm<sup>3</sup> was 22%, and for those whose CD4 was < 350 cells/mm<sup>3</sup> prevalence was 67% (OR=7.05; 95% CI: 3.7-13.3,  $P<0.05$ ).

**Conclusion:** BV is more prevalent among HIV-positive pregnant women than their HIV-negative counterparts, and its occurrence is higher among those with the lower CD4 count; therefore, reducing the prevalence of HIV among pregnant women and improving the CD4 count among HIV-positive pregnant women would reduce the prevalence of BV and its consequences. (Int J Biomed. 2015;5(3):141-146.)

**Keywords:** Bacterial Vaginosis; HIV transmission; Amsel's criteria.

### Introduction

BV is a common vaginal infection that involves the disruption of the balance of bacterial flora in the vagina with the overgrowth of specific bacteria. Normally, bacteria belonging to the *Lactobacillus* type live in the vagina as commensals. In BV, however, increased numbers of bacteria, such as *Gardnerella vaginalis*, *Bacteroides spp*, *Mobiluncus spp*, *Prevotella spp* and *Mycoplasma hominis*, replace them [1]. The reported prevalence of BV in pregnant women ranges from 14% to 21% in Western countries [2-4], and a prevalence rate of 40.8% was reported in a non-pregnant population in southern Nigeria [5]. A case study done in Ghana showed a

low prevalence of 1.4% among antenatal women [4]. An estimated 25% to 30% of women have BV at any time, mostly without signs such as fishy odor or discharge [1,4], and this rises to 85% in prostitute populations [2,3].

A study of pregnant women demonstrated that women who had BV on screening were 5 times more likely to have preterm labor or late miscarriage than those without the condition [5]. In women delivered by Caesarean section, those with BV were nearly 6 times more likely to develop postpartum endometritis than those without BV despite antibiotic prophylaxis, and BV was the strongest predictor of postpartum endometritis irrespective of mode of delivery [6]. Of greater clinical importance is the link between BV and HIV infection. Apart from its association with adverse pregnancy outcomes, BV is associated with increased risk of sexual acquisition of HIV [1,3,8]. There is evidence to support several mechanisms through which BV and other reproductive tract infections facilitate HIV transmission [7]. Genital tract

\*Corresponding author: Adelaiye, Samuel Makanju (MB. BS, PGDCS, FWACS). Federal Medical Centre, Azare, Bauchi State, Nigeria. Email: [samueldelaiye@yahoo.com](mailto:samueldelaiye@yahoo.com)

infection with *G. vaginalis*, which is commonly isolated in BV, has been shown to be able to stimulate HIV-1 reproduction and hence increase the likelihood of sexual transmission [8]. Measurement of HIV in genital secretions indicates that HIV infectiousness and susceptibility may be greater in the presence of concurrent reproductive tract infections [8,9]. Also significant is the sharp decline in the concentration of HIV in genital secretions when the infection is treated [1,2,8].

Several cross-sectional studies performed in Thailand, Uganda, and Malawi, showed that BV women had an increased incidence of HIV infection. A prospective study in Kenya also showed that the presence of BV and the absence of lactobacilli or absence of hydrogen peroxide-producing lactobacilli upon examination, were all significantly associated with acquisition of HIV infection at follow-up [1,2]. Studies of the impact of BV on HIV infections showed varying degrees of increased risk of infection [2]. Some investigators calculate that in a population where the baseline risk of seroconversion is 2%, one additional case of HIV infection would occur for every 80 to 90 cases of vaginosis. “These data suggest that greater attention needs to be given to bacterial vaginosis in the global fight against HIV infection” [1].

In view of the relationship between BV and HIV infection and also the associated risk of adverse obstetric sequelae, including increased vertical transmission of HIV during pregnancy, screening women for BV and treating those infected is a major strategy in the primary and secondary prevention of HIV as well as reducing risk of mother-to-child transmission of HIV, particularly in settings of high HIV seroprevalence.

**The aim and objectives**

The aim of this study was to find the characteristics of BV among HIV-infected and HIV-negative pregnant women at ABUTH, Zaria.

The specific objectives are to:

- Determine the prevalence of BV among HIV-infected pregnant women
- Determine the prevalence of BV among HIV-negative pregnant women
- Compare the prevalence of BV in the two groups
- Ascertain any relationship between the presence of BV and the immunological status of HIV-infected pregnant women
- Derive recommendations relevant to BV that will benefit the study population, and could be scaled up

Null Hypothesis: There is no statistically significant difference in the prevalence of BV between pregnant women who are HIV-positive and those who are HIV-negative in the same clinical setting.

**Materials and Methods**

**Study area and design**

The study was case controlled in design and involved both clinical and laboratory- based components. The clinical segment of the study was carried out simultaneously at the

PMTCT and antenatal clinics of ABUTH, while the laboratory analyses were made at the AIDS Prevention Initiative in Nigeria (APIN)/Harvard/PEPFAR supported laboratory of ABUTH, Zaria..

The study participants were selected from the antenatal clinic after obtaining informed written consent for participation in the study.

The sample size for the study was determined using the formula below, and BV prevalence among HIV-negative pregnant women of 14% [1-4].

$$N = \frac{Z^2 pq}{D^2} , \text{ where}$$

- N- Minimum sample size
- Z- 1.96 at 95% confidence level
- P - Prevalence of the condition
- D- Level of significance, 0.05 at 95% confidence level

This gave a minimum sample size of 185 for each group. A 10% attrition rate was 18.5; this was approximated to 15, giving a total of 200 for HIV-positive pregnant women and 200 for HIV-negative pregnant women as controls for the study.

For every HIV-infected patient recruited at the PMTCT clinic, an HIV-uninfected patient of similar age, parity, and socioeconomic status was recruited as control from the ANC until the total number of 200 was obtained in both groups (Table 1 and 2). Using convenience sampling technique, we selected consecutive pregnant women who consented and met the inclusion criteria.

**Table 1.**

**Age and parity of both groups’ patients**

Age group	Parity of HIV-positive pregnant women			Parity of HIV-negative pregnant women			Total
	0	1-4	≥5	0	1-4	≥5	
15-19	3	4	-	3	4	-	14
20-24	14	30	-	14	30	-	88
25-29	12	44	2	12	44	2	116
30-34	8	31	10	8	31	10	98
35-39	4	14	15	4	14	15	66
≥40	-	6	3	-	6	3	18
Total	41	129	30	41	129	30	400

Those diagnosed with BV were treated free-of-charge using tabs Metronidazole 400 mg, 8 hourly, for 7 days.

The following criteria were used to select the participants for the study:

- Informed consent to participate in the study
- Those women who had gone through HIV pre-test counseling and testing (HCT) by ABUTH PMTCT

nurse counselors

- Those who were reported as HIV-positive after undergoing the double rapid serial testing according to the National HIV Testing Algorithm and received post-test counseling
- Pregnant women who were reported as HIV-negative by double rapid serial testing and received post-test counseling were recruited as controls (matched for age and parity).
- The diagnosis of BV was based on the Amsel diagnostic criteria (Table 3) [9].

**Table 2.**

**Socio-demographic data of patients**

Variable	HIV- positive		HIV-negative		Total
	n	%	n	%	
<b>Marital Status</b>					
Married	198	49.7	200	50.3	398
Single	-	-	-	-	
Divorced	-	-	-	-	
Widowed	2	100	0	0	2
<b>Highest Educational Attainment</b>					
Primary	22	68.8	10	31.2	32
Secondary	70	53.0	62	47.0	132
Tertiary	74	40.0	111	60.0	181
Quranic	28	62.2	17	37.8	45
None	6		4		10
<b>Marriage Order</b>					
First	122	39.1	190	60.9	312
Second	74	91.3	7	8.4	81
Third	4	57.1	3	42.8	7
<b>Marriage Type</b>					
Monogamy	140	47	158	53.0	298
Polygamy	60	58.8	42	41.2	102
<b>Total Number of Partner over time</b>					
1-2	100	50.2	99	49.8	199
3 or more	76	51.7	71	48.3	147
Missing:54(13.5%)					

**Table 3.**

**The Diagnosis of BV based on the Amsel diagnostic criteria**

Laboratory findings	HIV-positive		HIV-negative	
	positive n (%)	negative n (%)	positive n (%)	negative n (%)
Whiff test	108 (27%)	88 (22%)	102(25.%)	80(20%)
PH (> 4.5)	102(26%)	100(25%)	132 (33%)	76 (19%)
Clue cells	125(31.2%)	92 (23%)	58(14.5%)	73(18.2%)
V. discharge	106(26.5%)	142(35.5%)	56 (14%)	137(34.2%)

Approval for the study was obtained from the ABUTH Ethical Review Committee. Doctor and nurse interviewers who were specifically trained for the study screened each woman for eligibility, explained the purpose and practice of the study and obtained informed written consent, which was written in English and translated for those who could not read English.

The instrument used was a pre-tested, semi-structured

proforma. It was used to collect socio-demographic information, medical history, HIV infection characteristics, clinical “bedside” test results, and BV laboratory findings. Trained nurses and the author administered the questionnaire. The clinical “bedside” test was done by the author, and other information, such as CD4 count, was obtained from the patients’ records. The laboratory scientist filled in the laboratory (clue cells) findings

The data assembled on the questionnaires were cleaned. A database was developed using SPSS 16.0 version for Windows and data entry was made into it. Further data cleaning was done before analysis was commenced, and relevant tables and graphs were generated where appropriate. The chi-square test was used to determine the strength of association between exposure and outcome variables in the experimental and controlled groups. A probability value of  $P < 0.05$  was considered statistically significant.

Specimen materials for making laboratory diagnosis were collected during a comprehensive pelvic examination. Following adequate counselling, each subject was placed in the dorsal position on an examination couch in the clinic. Using a sterile disposable Cusco speculum to expose the cervix and posterior fornix, the clinician evaluated the nature of the discharge, and a specimen from the posterior fornix was taken with a sterile swab stick. The classical BV discharge is characterized by a thin, homogeneous discharge, grey/yellow in colour. However, absence of the classical discharge did not rule out disturbed vaginal flora. Two basic methods of diagnostic testing were used: laboratory-based and clinical ‘bedside’ testing. For the purposes of laboratory-based testing, the swab was taken from the posterior vaginal fornix, diluted with normal saline and allowed to air dry, then kept in a slide rack which was used to transport the slides to the laboratory where they were gram stained and examined under the microscope (X400).

**Diagnosis of BV by Amsel Criteria (Amsel et al., 1983)**

The pH of the vagina was determined directly with the use of Combi 9 pH sticks placed on the posterior-vaginal fornix, after exposing the cervix with a sterile Cusco speculum that was inserted into the subject placed in the dorsal position. A swab stick was used to obtain a specimen which was then extracted into 0.2 mL of physiological saline on a glass slide. A drop of 10% potassium hydroxide was placed on another glass slide. The swab was then stirred in the 10% potassium hydroxide and immediately evaluated for the presence of a fishy odor. The extracted drop was placed in a slide rack and allowed to air dry. The slides were transported to the laboratory for examination, for detection of clue cells by Gram stain microscopy, which was examined under X100 objective for clue cells, at ART laboratory of the Ahmadu Bello University Teaching Hospital, Zaria.

**Limitation of the study:** a) The fact that Amsel’s criteria used 3 of 4 diagnostic criteria made diagnosis very cumbersome; b) The unavailability of a single diagnostic test made the study unduly expensive; c) Matching of the patient for age parity was difficult; We had to obtain the sample on different days.

## Results

1. The 200 HIV-negative pregnant women that were ultimately recruited as controls in this study were secured from 239 eligible women: 15 were dropped during the process of matching for age and parity of the study group; 15 failed to return/complete the consent forms, and another 9 were replaced after their specimens encountered a number of mix-ups in the laboratory. To secure the 200 sample size of HIV-positive pregnant women, a total of 209 eligible women were recruited, with the following attrition: 3 women failed to return/complete the consent forms, 4 had wrong numbering of specimens in the laboratory, and 2 incurred broken slides, also in the laboratory.

This study was conducted between August 2 and December 30, 2011, during which period a total of 757 new antenatal attendees were seen in the facility where the study was carried out. Out of these 98 were HIV-positive, 7 of whom were newly diagnosed with HIV. This gave a clinical HIV prevalence of 12.9% during the study period. The overall prevalence rate of BV in this study irrespective of HIV status was 33.2%.

2. Prevalence of BV among HIV-positive pregnant women was 46.0% (Table 4).

**Table 4.**

**Prevalence of BV and HIV status of pregnant women at ABUTH Zaria**

HIV status	BV				
	positive n (%)	negative n (%)	P	OR	95% CI
HIV-negative	41(20.5%)	159(79.5%)		1.0	
HIV-positive	92(46.0)	108(54.0%)	0.000	3.30	2.1-5.1
Total	133(33.2%)	267(66.8%)			

In this HIV group, BV was more commonly associated with tribe, religion, and parity, with *P*-values of 0.001, 0.03 and 0.04, respectively, which were all statistically significant. Other factors that did not show any statistical significance were level of educational attainment, order of marriage, type of marriage, number of partners over time, age of the subject, occupation, and gestational age, with *P*-values of 0.07, 0.62, 0.36, 0.22, 0.06, 0.28 and 0.055, respectively, as their *P*-values were  $>0.05$ .

3. BV prevalence among pregnant women who were HIV-negative was 20.5%

In those who were HIV-negative, BV appeared to be more commonly associated with level of educational attainment and the age of the subject, with *P*-values of 0.024 and 0.025, respectively, which were both statistically significant. Other factors that did not show any statistical significance were order of marriage, type of marriage, number of partners over time, tribe, religion occupation, gestational age, and parity, with *P*-values of 0.25, 0.28, 0.31, 0.29, 0.40, 0.34, 0.49 and 0.29, respectively, as their *P*-values were  $>0.05$ .

4. The odds of occurrence of BV in HIV-infected women

was 3 times higher than among HIV-uninfected women (OR=3.30; 95% CI: 2.1-5.1,  $P<0.05$ ).

5. The pattern of BV prevalence among those who were HIV-positive at various levels of CD4 count were as follows: For those with CD4  $> 350$  cells/mm<sup>3</sup> the prevalence rate was 22%, and for those with CD4  $< 350$  cells/mm<sup>3</sup> the rate was 67%. The odds of BV occurrence in HIV-infected women with CD4 count of  $\leq 350$  cells/mm<sup>3</sup> was 7 times higher than in HIV-infected women whose CD4 count was  $> 350$  cells/mm<sup>3</sup>, and this was statistically significant (OR=7.05; 95% CI: 3.7-13.3,  $P<0.05$ ) (Table 5).

**Table 5.**

**The pattern of BV among HIV- positive pregnant women and their CD4 count**

CD4 cells/mm <sup>3</sup>	BV				
	positive	negative	P	OR	95% CI
$\leq 350$	71(67.0%)	35(33.0%)		1.00	
$> 350$	21(22.3%)	73(77.7%)	0.000	7.05	3.7-13.3
Total	92(46.0%)	108(54.0%)			

## Discussion

This case-control study was done to determine the prevalence of BV among HIV-positive antenatal women, compared to their HIV-negative counterparts, and to relate the presence of BV to their immunological status at ABUTH, Zaria.

The prevalence of BV in both case and control group was found to be 33.2%. The prevalence of BV among HIV-infected pregnant women in this study was 46% compared to 20.5% among HIV-uninfected women. This difference is statistically significant (OR=3.30,  $P=0.000$ ), thus suggesting that HIV is a significant exposure risk for BV. Therefore, some HIV clinics also provide sexual health screens and test for BV as part of their care [10].

Demographically, in the HIV-positive pregnant group, BV was more commonly associated with tribe, religion, and parity. Tribe and religion may be associated with socio-cultural habits, including risk practices that may include douching with vaginal washes or soaps; other things inserted in the vagina erroneously include tampons, pads, powders and sprays. The use or non-use of contraceptives and the type might affect the prevalence of BV: hormonal contraceptives are protective of BV [8,11] while intrauterine contraceptive devices have been associated with increased susceptibility to BV [10,12]. These are all dependent on tribal and religious beliefs [12]. The prevalence of BV was highest among multiparous (1-4) women (54%) compared to nulliparous women, and this was statistically significant. This could be due to the fact that multiparous women are more likely to have had more sexual intercourse, which is a known risk factor, and also because of the drop in cellular immunity in this group of patients [8,13]. Parity was not a significant factor among the HIV-negative group.

Demographically, in the HIV-negative group, BV was

more commonly associated with the level of educational attainment and the age of subject. It was higher among those with no form of education. Low education has been linked to higher BV-risk in the literature [14]. It was more prevalent in the age group 30–34 years (37.8%), which was significant. This could be due to the fact that this age group is in the active reproductive period and patients are more sexually active. Other studies showed a high prevalence in the age group 25 to 29 years [14,15].

Demographically, in both groups, BV was more prevalent in the second trimester though this was not statistically significant. This could be as a result of late booking: mean gestational age at booking was 20 weeks. All the women studied were married except two that were widowed. Though the prevalence of BV was equal among women in monogamous and polygamous unions, women who were BV-negative were more likely to be in monogamous marriages than in polygamous marriages. This could be explained by the fact that those in monogamous settings are less likely to have multiple sexual partners than their polygamous counterparts. Having multiple sexual partners has been identified as a risk factor [10,16,17], although the number of partners over time was not significantly associated with BV. Many (13.5%) were too shy to give information on their number of partners, considering its sensitive nature, especially in northern Nigeria where this study was conducted.

In HIV-positive pregnant women, considering one of the criteria for instituting HAART (CD4 count), among those with CD4 count  $\leq 350$  cells/mm<sup>3</sup>, the prevalence of clinical BV was 67%. This was 7 times higher than in those who had CD4 counts greater than 350 cells/mm<sup>3</sup>, in whom the prevalence rate was 22.3% and was similar to the prevalence rate of 20.5% among HIV-uninfected pregnant women. It is possible that HIV infection alters the local immune mechanism in the lower genital tract, which puts those women with HIV at higher risks for the BV organism to thrive and proliferate in the vagina [8,17]. Furthermore, studies have shown that HIV load in the genital tract correlates positively with BV and inversely with the absence of BV [1,12,17]. The increase in HIV load in the genital tract of BV women leads to more exposure to this high viral load during the process of labor and delivery, thereby increasing the risk of mother-to-child transmission of HIV if this infection is not identified and treated in pregnancy [1,2]. These results, therefore, imply that identification and treatment of BV is an important strategy in the prevention of HIV transmission, particularly with respect to mother-to-child transmission.

Cell-mediated immunity is an important component of protection against genital tract pathogens; the higher the number of memory T-cells the better protection against infections [2]. T-cells are the main target of HIV in the blood, and they act as the host the virus needs to replicate. One important feature of the T-cell's structure is the CD4 receptor site; once these "coordinator" T-cells are hijacked, the immune system is annihilated, leaving the body open to infections [2,17]. Therefore, the CD4 count is a good reflector of the immune system; hence the lower the CD4 count, the higher the prevalence of BV, as shown in this study.

To detect clue cells, the gram stained smear has been found to have higher sensitivity and specificity [11,12,15]. Despite this, none of the 4 criteria was as accurate as it should have been, hence the need for the composite criteria [14,18,19]. It is also possible that not all BV patients had the offending organisms diagnosed. This may be due to the use of antibiotics or antiseptic soaps or preparations for douching prior to presentation at the clinic [14,19].

## Conclusion

This is probably the first case control study in Nigeria that compared the prevalence of BV among pregnant women who are HIV-positive and their HIV-negative counterparts and that related the presence of BV to the immunological status of the HIV-positive antenatal group. The prevalence of BV infection among HIV-infected pregnant women is 3 times higher than among their HIV-negative counterparts. Likewise, the prevalence of BV is 7 times higher among HIV-infected pregnant women whose CD4 counts were  $< 350$  cells/mm<sup>3</sup> compared to those whose CD4 were  $>350$  cells/mm<sup>3</sup> in ABUTH, Zaria.

## Competing interests

The authors declare that they have no competing interests.

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