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EVALUATION OF BACTERIAL VAGINOSIS (BV) USING NUGENT SCORING SYSTEM

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Abstract

Bacterial vaginosis (BV) is the most common form of vaginal infection with diverse etiology in women of reproductive age. It may lead to morbidity and obstetric/gynecological complications. This study was carried out to determine the usefulness of Nugent scoring system as a means of diagnosing bacterial vaginosis. Sixty-seven (67) women between the ages of 16 and 45 years were enrolled in the study. Vaginal swabs were Gram stained and slides examined for Nugent scoring. BV was diagnosed in 13.4% of women and the highest prevalence found in the age group of 16-20 years. The relationships of a positive whiff test (P=0.0051), presence of clue cells (P=0.001) and a pH>4.5 (P=0.0077) with bacterial vaginosis were shown to be statistically significant. Nugent scoring system appears to be a reliable and convenient method for laboratory evaluation of bacterial vaginosis.

Introduction

Bacterial vaginosis (BV) is the most common vaginal infection resulting in discharge/discomfort in developing and industrialized countries. Several studies have shown strong bidirectional association between BV and an increased risk for acquisition of sexually transmitted diseases (STDs), including human immunodeficiency virus (HIV)/acquired immune deficiency syndrome (AIDS) [1, 2, 3]. A large number of gynaecological complications are also associated with BV [4]. The effect on pregnancy is detrimental leading to preterm delivery and low birth weight [5]. It is about twice as common as candidiasis [6]. The prevalence of BV among women varies considerably and in Nigeria about 14.2% of premenopausal women are documented to be affected by BV [7]. Various studies have found the prevalence of BV to range from 15 to 30 per cent in non-pregnant women and up to 50 per cent in pregnant women [8]. The prevalence of BV varies in different parts of the world, e.g., 25% in a group of healthy Canadian women [9], 29.9% in Indonesia [10], 15% in rural Brazil [11].

The etiological agents of BV is still debatable, however, a common denominator is a disturbance in the vaginal microbiota, with absence and or of anaerobic Gram-negative bacilli. BV is a condition involving a polymicrobial process with interrelated organisms leading to the nuisance and complications associated with it. Gardnerella vaginalis appears to be the only one among several bacterial genera or species that are more common or present in

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larger quantities in women with BV compared to healthy controls. Other organisms that have been incriminated include Mycoplasma hominis [12], Mobiluncus spp., Prevotella spp., Atopobium vaginae, Eggerthella spp., Megasphaera spp., Leptotrichia spp., Peptoniphilus spp., Anaerococcus spp., Dialister spp. and recently a novel bacterium that belongs to the order of Clostridiales [13]. The vaginal microbiota consists of principally Lactobacillus species in healthy women [14] and our recent genomic study has shown that Nigerian women are colonized with some Lactobacillus species with probiotic capabilities [15].

The two most widely accepted methods for the diagnosis of bacterial vaginosis are Amsel's composite criteria [16] and Nugent's Gram stain evaluation of bacterial morphotypes [17]. The clinical criteria of diagnosing BV is confirmed using the composite criteria described by Amsel et al., [16], in which 3 or 4 of the following have to be present for diagnosis; (1) A thin homogenous discharge. (2) Elevated vaginal pH above 4.5 (3) Release of amines on addition of 10 percent potassium hydroxide solution to vaginal fluid and (4) The presence of clue cells on wet mount. These are not used sufficiently in routine practice [18], mostly in low-income countries especially in Nigeria. The diagnosis of BV by Amsel criteria is simple, however, it is relatively insensitive and not easily subjected to quality control, and the apparent complexity of the latter may have limited its routine application by clinical laboratories without the expertise.

Although BV is not a new clinical condition rather it is an infection that is under recognized and misdiagnosed in developing countries including Nigeria. Due to this under recognition, most clinicians do not even recognize BV diagnosis as a routine laboratory investigation for females of childbearing age. This study was carried out to determine the usefulness of using the Nugent scoring system as a means of diagnosing BV in our environment especially at the University of Benin Teaching Hospital.

Materials and Methods

Study centre and exclusion criteria

The study was conducted at the University of Benin Teaching Hospital and School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Benin City, Edo state from June to September 2012. Sixty-seven (67) high vaginal swab samples were obtained from pre-menopausal women between the ages of 16 and 45. Women were enrolled after giving informed consent. Exclusion criteria included women in their menses and women on antibiotics.

Vaginal sample collection and examination

Samples were collected from the lateral vaginal wall of these women using two sterile cotton tipped swab sticks. A pH strip was used to measure the pH of the vaginal sample by simply touching vaginal swab with the strip and comparing the colour change against a narrow range pH reader. The swab was then smeared on a glass slide for Gram staining. This swab was immediately mixed with 2 drops of 10% KOH solution for amine testing (whiff test). The composite clinical diagnosis of BV was defined as presence of at least three of the following; Homogenous vaginal discharge, pH >4.5, positive whiff test and presence of clue cells. The smear on the glass slide was allowed to air dry after which it was heat fixed and Gram stained.

Evaluation of Gram stained smear

Vaginal smears were made on microscope slides from the vaginal swab collected from each subject. The slides were Gram stained and bacterial morphotypes determined by viewing five different fields, using the oil immersion objective (100x) and taking the average. Evaluation of the smear was made using Nugent criteria scores [17]. A score of 0-10 was assigned considering the relative proportions of large Gram-positive rods (lactobacilli), small Gram-negative or Gram variable rods (Bacteriodes, Prevotella, or Gardnerella species) and curved Gram-variable rods (Mobiluncus species). A score of 0-3 was interpreted as consistent with normal microbiota, a score of 4-6 as intermediate and a score of 7-10 was considered consistent with BV-like conditions.

Statistical analysis

The data obtained were subjected to statistical analysis by using GraphPad Prism software version 4 (GraphPad Software Inc., California, USA) Nonparametric statistical method, employing Pearson's Chi-Square and Cramer's V were used to test the association between the Amsel criteria, Nugent Score and BV. P value < 0.05 and Cramer's V close to 1 was considered significant.

Results

A total of 67 women consented to participate in the study from July to September 2012. The age range was 16-45 years with a mean age of 24.9 years. The demographic structure of participants showed that 46 (68.7%) were female students, 12 (17.9%) business women, and 9 (13.4%) civil servants. Table 1 shows the age distribution of participants with Nugent scores interpreted as normal (N), Intermediate (I) and BV cases. Normal microbiota was found in 43 (64.2%), Intermediate in 15 (22.3%) and BV in 9 (13.4%) of the women. Table 2 shows the association of BV with Gram Positive Cocci (GPC), Gram Negative Bacilli (GNB) and Candida. Table 3 shows the distribution of other criteria over the categories of patients with and without BV. Individuals with vaginal discharge accounted for 25.4% of the samples out of which 44.4% had BV. Clue cells were also found to be significant ($P=0.001$) and there was a high association of Clue cells with bacterial vaginosis (Cramer's $V=0.6796$). A positive whiff test and $pH>4.5$ were significant ($P=0.0051$ and $P=0.0077$ respectively) but there was a moderate association of whiff test and pH.4.5 ($V=0.3968$, $V=0.3811$ respectively).

Discussion

To our knowledge, this is the first study using Nugent scoring system to diagnose BV in the Department of Medical Microbiology Laboratory, University of Benin Teaching Hospital (UBTH). Over 80% of the participants have putative normal microbiota colonized with Lactobacillus morphotypes and this is in agreement as it is generally accepted that these bacteria form a critical line of defense against potential pathogens. The symbiotic relationship between vaginal lactobacilli and their human host is modulated by estrogen (the hormone circulating in premenopausal woman), which stimulates the vaginal epithelia to produce glycogen [18]. Vaginal lactobacilli metabolize glycogen secreted by the vaginal epithelia, in turn producing lactic acid, which is largely responsible for the normal vaginal pH being acidic (<4.5) [20]. The highest prevalence of BV was found in the age group 16-20 years with a percentage of 33.3. This age group usually engage in activities that may increase their risk, such as cigarette smoking, douching, antibiotic treatment for another condition, young age of sexual intercourse [21], acquisition of a new

Table 1: Age distribution showing Normal, Intermediate and BV cases.

Age group (years)	Normal (%)	Intermediate (%)	BV (%)
16-20	13 (30.2)	2 (13.3)	3 (33.3)
21-25	15 (34.9)	7 (46.7)	2 (22.2)
26-30	6 (13.9)	4 (26.7)	1 (11.1)
31-35	7 (16.3)	1 (6.7)	2 (22.2)
36-45	2 (4.7)	1 (6.7)	1 (11.1)
TOTAL (67)	43 (100)	15 (100)	9 (100)

Key: BV=Bacterial vaginosis

Table 2: Association of microorganisms with Nugent score interpreted as BV

MCO			**NUGENT SCORE**		
		N (%)	**NORMAL 0-3 (n= 43)**	**INTERMED 4-6 (n= 15)**	**BV 7-10 (n= 9)**
GPC	Positive	14 (20.9)	9 (20.9)	3 (20.0)	2 (22.2)
	Negative	53 (79.1)	34 (79.1)	12 (80.0)	7 (77.7)
	?²=0.02, *P*=0.99; Cramer's V=0.0173				
GNB	Positive	15 (22.4)	4 (9.3)	6 (40.0)	5 (55.5)
	Negative	52 (77.6)	39 (90.7)	9 (60.0)	4 (7.7)
	?²=12.61, *P*=0.0018; Cramer's V = 0.4338				
Candida	Positive	14 (20.9)	11 (25.6)	3 (20.0)	0 (0)
	Negative	53 (79.1)	32 (74.4)	12 (80.0)	9 (100)
	?²=2.96, *P*=0.2276; Cramer's V= 0.2102				

Key: **GPC**: Gram-positive cocci, **GNB**; Gram-negative bacilli, **BV**: Bacterial vaginosis

Table 3: Comparison of Amsel criteria with Nugent scores showing normal, intermediate and bacterial vaginosis.

Amsel criteria		N (%)	NORMAL 0-3 (n= 43)	INTERMED 4-6 (n= 15)	BV 7-10 (n= 9)
Clue cell	Positive	15	1 (2.3)	7 (46.7)	7 (77.8)
	Negative	52	42(97.7)	8 (53.3)	2 (22.2)
	$?^2$=30.94; *P*=0.0001; Cramer's V = 0.6796				
Whiff test	Positive	16	7 (16.3)	3 (20)	6 (66.7)
	Negative	51	36 (83.7)	12 (80)	3 (33.3)
	$?^2$=10.55; *P*= 0.0051; Cramer's V = 0.3968				
Discharge	Positive	17	12 (27.9)	1 (6.7)	4 (44.4)
	Negative	50	31 (72.1)	14 (93.3)	5 (55.6)
	$?^2$=4.65; *P*= 0.0978; Cramer's V = 0.2634				
pH > 4.5	Positive	28	14 (32.6)	6 (40.0)	8 (88.9)
	Negative	39	29 (67.4)	9 (60.0)	1 (11.1)
	$?^2$=9.73; P=0.0077; Cramer's V= 0.3811				

Key: $?^2$=Chi square

sex partner and a recent history of multiple sex partners have been found to be associated with high rate of BV [22]. The lowest prevalence was observed in the age group 36-45 years with a percentage of 11.1% (Table 1). This age group is matured and may probably not get themselves involved in high-risk activities. Table 2 shows the association of BV with other organisms, indicating the distribution of Gram positive cocci (GPC), Gram negative bacilli and presence of yeast cells as detected by microscopy. About 50% of individuals with Gram negative bacilli were found to have BV, this shows that there is a high association between BV and Gram negative bacilli ($P=0.0018$). The occurrence of Gram negative bacilli have been found previously to be associated with BV [23]. Another study by Spiegel [24] described appearances of Gardnerella morphotypes along with other small Gram-negative rods and Gram-positive cocci, to be associated with BV, in contrast to this study where the presence of GPC was found to have no association with BV.

Our study is in congruent with the diagnosis of BV in clinical settings based on fulfilment of three of four clinical criteria

described by Amsel et al. [16]. Amsel's criteria includes (i) elevated vaginal pH (>4.5), (ii) presence of white adherent discharge that contains (iii) numerous exfoliated epithelial cells with bacteria (Gram-variable polymorphic rods) attached to their surface (clue cells) and (iv) has a characteristic fishy odour especially when 10% KOH is added. However, an estimated 25-30% women have BV at any given time, mostly without signs such as fishy-odour or discharge [25], and this rises to 85% in prostitute populations [28]. Due to asymptomatic nature of BV in some women, difficulties in culturing the causative agents and lack of modern microarray-based identification of clinically relevant vaginal bacteria in relation to bacterial vaginosis [13], diagnostic method such as Nugent's scoring system [17] is more objective in diagnosing BV [27] in our environment as shown in the present study.

Conclusions

The significant depletion of Lactobacillus morphotypes in the vaginal samples in this study lends credence to our previous findings that BV is associated with absence or low number of Lactobacillus species. Our study suggests that a simple Gram stained smear of vaginal discharge, may be utilized to diagnose BV. The method of Nugent scoring system used in this study for the diagnosis of BV could be introduced and it would be suitable for medical microbiology laboratory at the university of Benin teaching Hospital (UBTH). This method has the potential to complement or confirm the clinician's evaluation of patients suspected of having BV.

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