



Evaluation of vaginal cytology through clue cell detection and Nugent's score in the laboratory diagnosis of Bacterial Vaginosis in women of reproductive age

Authors

Musumba Cleophas Obonyo¹, Paul M Kosiyo², Kyama Mutinda¹, Evans Rabala³

¹Department of Medical Laboratory Sciences, Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya

²Department of Biomedical Sciences, Maseno University, Kenya

Email: paulkosiyo@gmail.com

³Dept of Medical Laboratory Sciences, Masinde Muliro University of Science and Technology, Kenya

Email: eraballah@hotmail.com

Corresponding Author

Musumba Cleophas Obonyo

Jomo Kenyatta University of Agriculture and Technology

Email: cleophasmusumba@gmail.com, Cell number +254720644513

Abstract

Background: Diagnosis of various causes of vaginal discharge is done by detailed history taking, physical examination and laboratory investigations. Use Nugent's score as laboratory tests remains the gold standard however, its use presents challenges in resource limited set ups. Bacterial vaginosis (BV) result from supplanted *Lactobacillus* species leading to disruption in the normal vaginal microbiome.

Objective: The main aim of the study was to evaluate vaginal cytology through clue cell detection and Nugent's score in the laboratory diagnosis of BV in women of reproductive age. Specifically, the study determined the association between vaginal pH and the quantity of clue cells in BV, and the correlation between vaginal pH and polymorphonuclear cell in BV among non-pregnant women of reproductive age residents this Kisumu County.

Methodology: The study was done at Jaramogi Oginga Odinga Teaching and Referral Hospital in 238 female participants aged (15-49yrs) presenting with vaginal discharge at Family Planning and Out Patient Department clinics. Clinical examination was done and laboratory specimens were collected to investigate the causative pathogens of vaginal discharge. Nugent's score was performed to detect bacterial morphotypes and clue cell in BV using one swab while the other swab was used for high vaginal swab examination was performed using wet mount technique.

Results: Results revealed positive correlation between clue cells and vaginal pH ($\rho=0.597$, $P<0.001$). At the same time women without BV [mean (SEM), 4.32 (± 0.05)] had significantly low vaginal pH relative to those with BV [mean (SEM), 4.90, (± 0.05)], $P<0.001$. Moreover there was, positive correlation between vaginal pH and PMNs ($\rho=0.271$, $P<0.001$).

Conclusion: Clue cell detection and vaginal pH are the most useful criteria in the diagnosis of BV among symptomatic patients in resource limited settings as confirmed by the determined associations. However, PMNs should not be considered a criterion in diagnosis of BV even if it correlates well with vaginal pH.

Keywords: Bacterial vaginosis, Nugent's score, pH, Gram stain, Normal flora

Background

Bacterial vaginosis is a condition which result from disturbance in the normal bacterial flora of the vagina^[1-3] and always accompanied by vaginal discharge in symptomatic stage. It is a regular occurrence in women of reproductive age and commonly observed by clinicians in plentiful medical institutions. BV is characterized by a shift in the vaginal ecosystem^[4]. It is a considered a polymicrobial clinical syndrome resulting from replacement of the normal hydrogen peroxide producing Lactobacillus species in the vagina with high concentrations of anaerobic bacteria^[4]. Several studies have demonstrated the relationship between Gardnerella vaginalis and other bacteria in causing BV^[5,6]. Such bacteria include Lactobacillus, prevotella, and anaerobes, as well as Mobiluncus, Bacteroides. The amount of vaginal discharge ordinarily present in the adult is such that the introitus feels comfortably moist; however, this is not enough to stain the underclothing. It is normally increased to the extent of becoming noticeable at the time of ovulation when there is 'ovulation cascade' from the cervix; during pregnancy when there is an increase in vaginal and cervical discharges^[7]. As much as the genital area should be kept clean, excessive cleaning i.e. use of disinfectants and perfumes disturb the vaginal flora leading to colonization of pathogens causing infection^[3, 8]. If left untreated, vaginal discharge can cause adverse consequences to maternal and child well-being, especially those of sexually transmitted infections (STIs) origin.

The prevalence of BV in different populations around the world ranges between 10–30%^[4] making BV the most common health problem affecting women. A previous study by Marrazzo and colleagues, revealed that the prevalence of BV among women who have sex with women as 29%^[14] Bacterial vaginosis is one of the most prevalent urogenital tract infection that prompts discharge among women of reproductive age^[9]. It is furthermore associated with about 60% elevated rise in risk of HIV-1 infection among women^[10].

Moreover, it is characterized by 3-62 fold increased risk of female to male HIV-1 transmission^[11]. The highest bacterial vaginosis and HIV burden is in Sub-Saharan Africa yet few studies have focused on etiology and laboratory diagnosis in this region. High prevalence of BV in Sub-Sahara Africa is approximately 55% hence this could be an important contributing factor high prevalence of HIV infection in this region^[11]. Previous studies have reported that BV increases the risk of acquiring HIV^[3,8,12] and intravaginal practices are an important risk factor for developing BV^[8] hence its early detection should be of a major priority. BV is characterized by overgrowth of anaerobes and decrease in the Lactobacillus whose function is to maintain the vaginal pH acidic for proper functioning^[13]. BV has been attributed to adverse obstetric outcomes including preterm labour and delivery, premature rupture of membranes, and spontaneous abortion as well as low birth weight^[14]. Most vaginal discharge patients are reported to have BV positive.

Diagnosis of various causes of vaginal discharge is done by detailed history taking, physical examination and laboratory investigations^[12]. The most accurate method for diagnosis is by laboratory a test which embraces exfoliative cytology and microbiology which is not readily available or unaffordable to most people in the developing world which is resource constrained^[15]. Several techniques have such as Nugent's score, Amsel criteria and Quick View Advance pH have been used to diagnose BV. Among all these techniques, Nugent's score has remained the gold standard but it is more involving, requires a lot of expertise and experience from the laboratory personnel and a challenge to execute in a resource limited set ups for a definitive diagnosis^[15-18]. We therefore in the current study evaluated the association between vaginal pH and the quantity of clue cells in BV, correlation between clue cells and BV using Nugent's score. In addition the study determined correlation between the vaginal pH

and polymorphonuclear cell in vaginal discharge among non-pregnant women of reproductive age from attending clinics at Jaramogi Oginga Odinga Teaching and referral hospital, Kisumu County Kenya.

Material and Methods

In this cross sectional study enrolled, a total of 238 consenting non-pregnant women of reproductive age (15 -49 years) attending different clinics JOOTRH (family planning, post natal and STIs) at JOOTRH in Kisumu County western part of Kenya. Sampling was conveniently conducted from July 2016 to May 2017. High vaginal swabs were collected by a qualified clinician during the hospital visits among women presenting with vaginal discharge to test for BV, vaginal candidiasis and Trichomonas vaginalis. Amount, odor and color of vaginal discharge were noted as major signs. Two vaginal swabs were collected simultaneously and instantaneously used as follows: one for Amsel scoring, while the second one was smeared onto clean microscope slide and taken to the laboratory where they were stained for Nugent' s scoring.

Ethical consideration

Interviews of all the participants were conducted by trained and qualified medical personnel identified by the Principal Investigator and done in a language chosen by the participants. Written consent forms in a local language were provided to the participants. The protocol was initially approved by the Board of Postgraduate Studies (BPS) of Jomo Kenyatta University of Agriculture and Technology and finally approved by Jaramogi Oginga Odinga Teaching and Referral Hospital Ethical Review Committee (JOOTRH ERC).

Study Site: The study was conducted at Jaramogi Oginga Odinga Teaching and Referral Hospital (JOOTRH) in Kisumu County. JOOTRH is located within the headquarter of Kisumu County-Western Kenya and within Kisumu East Sub-county with a population of 150,124 in an area of 135.90 km² [16]. Kisumu county has a population of 968,909 in an area of 2085.9 km² [16]. It has

seven sub counties namely Kisumu East, Kisumu West, Kisumu Central Muhoroni, Seme, Nyando and Nyakach. .

Study Population: This cross-Sectional study involved women aged 15-49 years hailing from the catchment area of Kisumu County between month of July 2016 to May 2017 and met the inclusion criteria described below during assessment of their eligibility. A questionnaire was used to collect demographic information from the participants.

Inclusion criteria: Residents in the study area who were non-pregnant or non-menstruating women of reproductive age (15-49 years). The participants had not been on antibiotic medication in the past 4 weeks during recruitment into the study and had a vaginal discharge and were willing and able to sign the consent form.

Exclusion Criteria: Pregnant and menstruating women at the time of hospital visit were excluded in the study. Those women who had received antibiotics in the past 4 weeks to the time of study were excluded. Women with clearly known STIs e.g. gonorrhoea were not included in the study.

Laboratory procedures

Measuring the pH of the vagina and Whiff test
pH was measured with pH paper held with forceps and dipped into the vaginal discharge.

Amsel criteria: BV was equally assessed by Amsel criteria [17]. BV was considered to be positive/present on the basis of at least 3 of the following signs; pH>4.5, positive Amine Whiff test (refer to the below procedure), presence of 3-5 clue cells per high power field on wet mount microscopy and homogenous vaginal discharge

Amine Whiff test: This was conducted by evaluating the production of amine odour upon addition of 10% potassium hydroxide to the vaginal discharge on the glass slide.

Nugent's score: Each type of the three type of bacteria morphotype i.e. Lactobacillus,

Gardnerella, and Mobiluncus was graded on a scale of 1-4 (1+ is < 1 cell per field, 2+ is 1-5 cells per field, 3+ is 6-30 cells per field, and 4+ is >30 cells per field) upon Gram staining. In this criterion, Lactobacillus and Gardnerella were given scores between 0-4 but Mobiluncus was only graded from 0-2. Total scores were then calculated and used as follows: 0-3 (Normal), 4-6 (intermediate bacterial count), and 7-10 (bacterial vaginosis) [5].

Data analysis: SPSS[®] statistical software package version 23.0 (IBM SPSS Inc., Chicago, IL, USA) and Graph Pad prism software (Graph Pad software, Inc., San Diego Calif.) were used in statistical analysis. Chi-square (χ^2) analysis used to determine differences between proportions, while Student's t-test with Welsch correction was used to compare normally distributed data. Correlations between clue cells, polymorphonuclear cell and vaginal pH were done using Spearman correlation test. All statistical significance were based at probability value; $P \leq 0.05$.

Results

Laboratory, demographic, and clinical characteristics of the study participants

Cross-sectional study was conducted on outpatient non-pregnant women (n=238) aged 15-49 years presenting with/without vaginal discharge and have sexual partners. The participants were clinically stratified based on Bacterial vaginosis status upon laboratory diagnosis with Nugent's score techniques as the gold standard. A total of 65.1% (155) participants did not have bacterial vaginosis (Non-BV) while 34.9% (n=83) participants had BV. Based on circumcision status of the participant's sexual partner, 50.6% (n=42) of the participants with circumcised sexual partners had BV while 49.7% (n=77) did not have BV. On the other hand, 49.4% (n=41) of the participants whose sexual partners were uncircumcised had BV whereas 50.3% (n=78) did not have BV ($p=0.0970$) (table 1). Laboratory

characteristics of the BV and non-BV were compared using t-test (Mann-Whitney U test) i.e. pH which confirmed statistical significance ($p < 0.001$). The proportion of clue cells, polymorphonuclear cells and the three bacterial morphotypes were significantly different between the two clinical strata ($p < 0.05$) (table 1).

Association between clue cells and vaginal pH

Alteration in the population of vaginal flora is mainly affected by the pH and this hypothesis has been adequately supported by various literatures [1,18,19]. The current study therefore assessed association between vaginal pH and BV status. There was a positive correlation between the vaginal pH and BV status. Analysis was performed using Spearman correlation test ($\rho=0.477$, $P < 0.001$), Figure 1.

Correlation between the polymorphonuclear cells (PMNs) and vaginal pH:

PMNs were considered to be granulocytes (a type of white blood cells) of varying nuclear morphology which is usually lobed into three segments. Since these PMNs could appear in vaginal discharge, the study determined the correlation between the vaginal pH and the amount of PMNs per eye power field (HPF). Spearman correlation test revealed a positive correlation between vaginal pH and PMNs ($\rho=0.271$, $P < 0.001$), Figure 3.

Findings from the micrographs: Clue cells and various bacterial morphotypes of various gram variability were microscopically demonstrated specifically on Gram stained smears. From this study the most fascinating feature exhibited was clue cells. In this case, coccobacillary organisms were seen attached /appearing in clusters on the cell surface, making the border indistinct or stippled as seen in figure 4 (D). Furthermore, several bacteria that constitute vaginal microbiome such as Lactobacillus, Gardnerella, and Mobiluncus were verified (Figure 4 A, B and C).

Table 1 Laboratory, demographic, and clinical, characteristics of the study participants

Characteristics	Disease status		P-Value
	Non-BV (n=155)	BV (n=83)	
Partner Circumcised			
Yes	77(49.7)	42(50.6)	0.970 ^a
No	78(50.3)	41(49.4)	
RBCs n (%)	18 (11.6)	12 (12.0)	0.921 ^a
Speculum Discharge Exam n (%)	28 (18.1)	46 (62.2)	<0.001 ^a
Vaginal pH, mean (SEM)	4.32 (0.05)	4.90 (0.04)	<0.001 ^b
PMNs/HPF	12 (7.7)	12 (14.5)	0.001 ^a
Clue cells/HPF	1 (0.6)	75 (90.4)	<0.001
Sperm cells	5 (3.2)	6 (7.2)	0.161 ^a
Epithelial cells	151 (97.4)	83 (100.0)	0.141 ^a
Pathogens identified			
T. vaginalis	8 (5.2)	8 (9.6)	0.189 ^a
Bacilli	7 (4.5)	63 (75.9)	<0.001 ^a
Yeast cells	20 (12.9)	8 (9.6)	0.456 ^a

Data presented for non-BV (n=155) and BV (n=83). Data are presented as n (%) unless stated otherwise. ^a Statistical analysis performed using Chi-square (χ^2) analysis; ^b Statistical significance was determined using student t-test with Welsch correction at $P \geq 0.005$. Abbreviations RBCs; Red Blood Cells; PMNs; Polymorph mononuclear cells; BV: Bacterial Vaginosis. HPF; High Power Field.

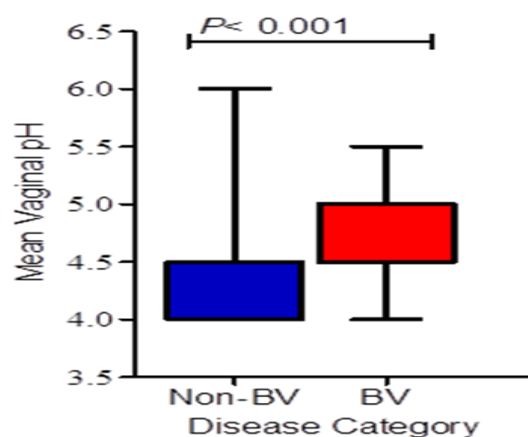


Figure 2: Differences between vaginal pH and BV status. Those with BV had higher vaginal pH compared to the non-BV ($P < 0.001$). Analyses done using Mann-Whitney U test at $P \leq 0.05$.

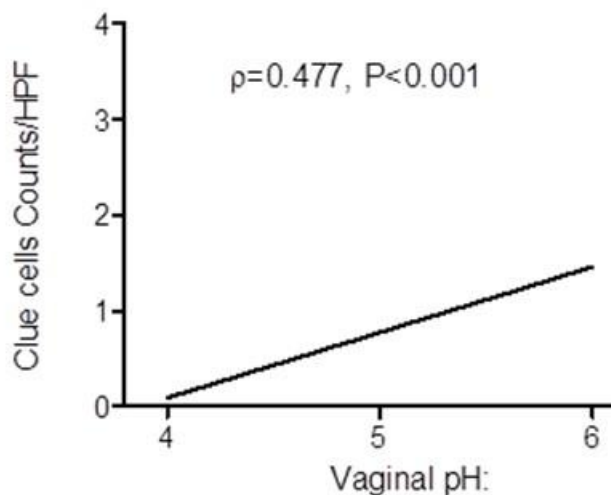


Figure 1 Correlation between clue cells and vaginal pH. This correlation was determined using Spearman correlation test. There was positive correlation between the clue cells and the vaginal pH ($\rho = 0.477$, $P < 0.001$).

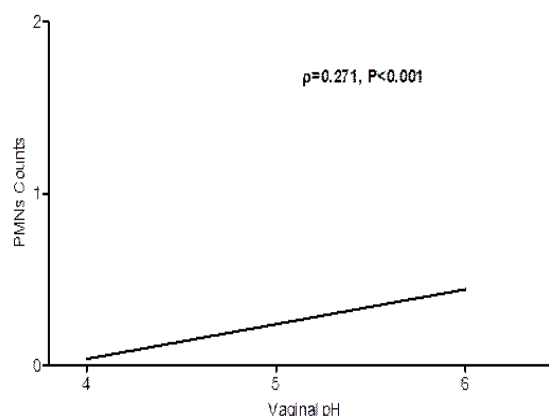


Figure 3: Correlation between PMNs and vaginal pH. The correlation was performed using Pearson correlation test at $P \leq 0.05$. There was increase in vaginal pH with increase in number PMNs ($\rho = 0.271$, $P < 0.001$).

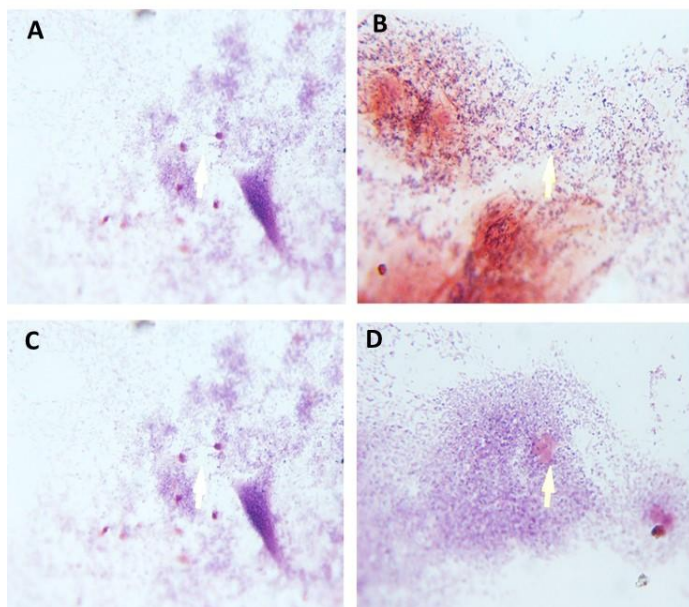


Figure 4: Micrograph of a Gram stained smear showing bacterial morphotypes: Lactobacillus and Gardnerella are Gram variable appearing dark to light purple while Mobiluncus spp. appear thin and pink in figures 4A, 4 B and 4C. Figure 4 D is micrograph of a Gram stained smear showing coccobacillary organisms attached /appearing in clusters on the cell surface, making the border indistinct or stippled. There is a clear demonstration of clue cell in BV (Nugent's Score=8) as shown by the arrow.

Discussion

It is generally acceptable that bacterial vaginosis is a serious health complication in women of reproductive age. The main objective of our study was to evaluate vaginal cytology through clue cell detection and Nugent's score in the laboratory diagnosis of bacterial vaginosis in women of reproductive age. As such, we recruited women aged (15-49 year presenting with vaginal discharge in the facility. In this respect, the study determined the association between vaginal pH and the quantity of clue cells in bacterial vaginosis, correlation between clue cells and BV and determined the correlation between the vaginal pH and polymorphonuclear cell in vaginal discharge among non-pregnant women of reproductive age. To our knowledge, this is the first study to report the correlation between vaginal pH and the quantity of clue cells per high

power field in evaluating diagnostic tools for bacterial vaginosis. A shift in vaginal pH above 4.5 favored BV because Lactobacilli which are hydrogen-peroxide (H_2O_2) producing appear to be important in preventing overgrowth of the anaerobes normally present in the vaginal flora. With the loss of lactobacilli, pH increases and enormous overgrowth of vaginal anaerobes occurs [20,21]. These anaerobes especially Gardnerella vaginalis yield large amounts of proteolytic carboxylase enzymes, which break down vaginal peptides into a variety of amines that are malodorous, volatile and associated with increased vaginal transudation and squamous epithelial cell exfoliation, resulting in the typical clinical features observed in patients with BV [21, 22]. Furthermore, rise in pH also assists adherence of Gardnerella vaginalis to the exfoliating epithelial cells which eventually accounts for the appearance of clue cells [19]. A study in a population of low prevalence of BV had earlier showed correlation of high vaginal pH with BV which equally suggests that vaginal pH as a simple tool for the diagnosis of BV [21].

Clue cells were defined as an epithelial cell of the vagina with a distinctive stippled appearance by being covered with bacteria [6, 19], figure 4 (A). They were first described by Gardner and Dukes [23] in 1955 and were so named as they gave important 'clue' to the diagnosis of BV. The result presented here demonstrates that clue cell was very pronounced and captivating finding in participants with BV and was the most reliable single indicator for BV. This is consistent with a previous study [20]. However, demonstration and identification of clue cells requires on-site microscopy facility, trained personnel and time as compared to measurement of vaginal pH. Moreover, it is generally accepted that for a positive result, at least 20 percent of the epithelial cells on wet mount should be clue cells. This is the single most definitive predictor of BV [7]. This indicator of bacterial vaginosis is particularly that caused by *Gardnerella vaginalis*, a group of Gram-variable bacteria that causes vaginal and

cervical erosion which can be associated with excessive non-purulent vaginal discharge due to the increased surface area of columnar epithelium containing mucus-secreting glands^[19]. This was evident in Gram stained smears which confirmed the presence of Gram variable bacteria (Figure 4 (B) and (C) respectively). Additional evidence suggests that *Gardenerrela vaginalis* is the principle player in the pathogenesis of BV and the development of a biofilm might be an important component of this process, in addition to the gradual overgrowth of resident anaerobic vaginal flora^[19]. Evaluation of both clue cells and pH could be better than syndromic management protocols and easier to implement in resource limited settings relative to Nugent's score which is labour intensive and requires a lot of expertise among other laboratory based techniques. This finding is in agreement with Posner and Thurman^[3,24]. It is important to note that BV per se typically does not cause dyspareunia, dysuria, pruritus, burning, or vaginal inflammation.

Conclusion

Clue cell detection and vaginal pH are the most useful criteria in the diagnosis of BV among symptomatic patients in resource limited settings as confirmed by a positive correlation. However, PMNs should not be considered a criterion in diagnosis of BV even if it correlates well with vaginal pH.

Declarations

Competing Interest Statement: The authors have declared no competing interest.

Authors' Contributions: CM, PK, EM, and KM designed, carried out the study in the rural population and participated in the drafting of the manuscript. EM and PK performed the statistical analyses and participated in the drafting of the manuscript. All authors read and approved the final manuscript.

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Ethics: The study was approved by the Jaramogi Oginga Odinga Teaching and Referral Hospital (JOOTRH) Ethical Review committee.

Consent for Publication: Not applicable

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Abbreviations

BV	Bacterial vaginosis
HPF	High power field
JOOTRH	Jaramogi Oginga Odinga Teaching and Referral Hospital
pH	Potential hydrogen
PMNS	Polymorphonuclear cells
STIs	Sexually transmitted infection