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Original Article

Comparison of Vaginal Culture and Pap smear in the Diagnosis of Bacterial Vaginosis

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ABSTRACT

Background: Amongst the myriad of physiological and pathological conditions presenting as vaginal discharge, bacterial vaginosis is the most frequently encountered complaint in women of child bearing age, all over the world. It involves the replacement of normally predominant hydrogen-peroxide producing lactobacilli, by an overgrowth of anaerobic bacteria. We want to examine the diagnostic efficacy of Pap-Smear and vaginal culture in the diagnosis of bacterial vaginosis, while Amsel's clinical criteria is used as the gold standard

Methods: It was a descriptive study expanding over a period of 5 months, from January 2013 to May 2013, enrolling 150 patients, from the outpatient's department of lady reading hospital and Hayatabad medical complex, Peshawar. All patients who complained of vaginal discharge were eligible for study. Patients using antibiotics, vaginal suppositories as well as those who were pregnant were excluded from the study. All patients were subjected to simultaneous testing for Amsel's criteria, vaginal culture, and Pap-staining. Sensitivity, specificity, positive predictive value and negative predictive values were calculated for vaginal culture and Pap smear, with amsel's criteria being the gold standard.

Results: Sensitivity, specificity, positive and negative predictive values for culture was determined as 75%, 92.1%, 64.3% and 95.1%. Pap smear was found to be 62.5% sensitive, 93.7% specific, positive and negative predictive values being 65.2% and 92.9% respectively.

Conclusion: Out of these two tests, vaginal culture was labeled as the more sensitive test for the diagnosis of bacterial vaginosis.

Keywords: Bacterial Vaginosis (BV), Papanicolou Smear (Pap smear), Culture, Amsel's Criteria.

Introduction

Bacterial vaginosis (BV) is prevalent in 35% of women seeking medical advice for sexually transmitted infections[1]. BV causes symptomatic vaginitis in 20-50% of women. Other causes include vulvovaginal candidiasis (17-39%) and trichomoniasis (4-35%)[2]. The term vaginosis is used instead of vaginitis because of the absence of an evident inflammatory response[3]. This infestation involves the replacement of normally predominant hydrogen-peroxide producing lactobacilli, by an overgrowth of anaerobic bacteria[4]. 99% of women suffering from BV harbor gardnerella vaginalis while atopobium vaginae is present in 96%.

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Gardnerella vaginalis is said to be present in small numbers (<10^6cfu/ml) in asymptomatic women while a count of greater than 10^6 indicates symptomatic BV[5]. Risk factors for acquiring BV include tobacco smoking, IUCD (intrauterine contraceptive device), douching, more than one sex partners, young age at first intercourse and black ethnicity[4]. It was recently found through FISH (fluorescence in situ hybridization) analysis, that biopsy specimens from vagina of women with symptomatic BV showed a dense biofilm, in 90% of the cases. This biofilm comprises mainly of gardnerella vaginalis that is the initial colonizer, serving as scaffolding for other bacteria, a process called co-aggregation. Atopobium vaginae makes up 40 % of the biofilm mass. Other bacteria, the secondary colonizers including bacteroids, corynebacteria, lactobacilli, veillonella, ruminococci and streptococci are present in lesser numbers[6]. BV can be asymptomatic, but most often certain distinctive clinical features are present including increased

fluidity of vaginal secretions, vaginal pH greater than 4.5, production of a fishy smell on addition of KOH (potassium hydroxide), Clue cells i.e. vaginal epithelial cells coated by bacteria. Clue cells are desquamated cells from vaginal epithelial lining that have bacteria adhering to their surfaces. These cells, when desquamate; give rise to the classic clue cells [7]. BV increases the hazards of acquiring complications in both obstetric and gynecologic patients, such as onset of pre-term labor, premature delivery, infection of uterine membranes around the fetus and post-surgical endometrial infections[1]. BV has been entwined with acquiring sexually transmitted infections and increased risk of acquisition of HIV. The STI include infections with Chlamydia trachomatis, Neisseria gonorrhea and HSV 1 & 2. The latest doctrine explaining this causality implicates the absence of protective lactobacilli as responsible for acquiring these infections.[1] The diagnostic methods employed for BV include: amsel's criterion, techniques employing stains for diagnosis, bacteriological culture and other methods. Among the methods used in diagnosing BV in clinics, Amsel'scriteria is the most eminent. Out of the four constituent parts of amsel's criteria, if three are found positive in a patient, it gives a positive diagnosis. The four constituent parts include: 1.a thin vaginal discharge, 2. Vaginal pH > 4.5, 3. Production of fishy odor upon addition of KOH called a 'whiff test', 4. Vaginal epithelial cells coated with bacteria , called clue cells[8].

In BV diagnosis, gram staining classified the bacteria as: 1. Morphotypes similar to Gardnerella, these are gram-negative or gram-variable. 2. Morphotypes resembling lactobacilli, these are gram-positive rods. Recent studies suggest that it is possible to use Pap smear to diagnose BV[9]. Methylene blue is used in STD clinics to diagnose BV. Gardnerella vaginalis, the principal microorganism implicated in causation of BV is recoverable in more than 85-90% of women who demonstrate symptoms of BV, but is also recovered in more than 50% of women who do not exhibit obvious symptoms of the condition. Culture of Bacteroids, Peptostreptococcus species and Mycoplasma hominis has reasonable specificity but is insensitive and expensive. Other anaerobes such as Mobiluncus can hardly be recovered by bacterial culture.[10]

Other diagnostic tests include: ELISA (for detection of anti-hemolysin antibodies of Gardnerella vaginalis), PCR, quick Vuer advanced pH and amine test card for detection of BV. Recently a molecular test affirms VP III assay has become commercially available. BV blue system for detection of sialidase activity is also employed[1,5,11]. The scoring systems used in diagnosis include Spiegel criteria; Nugent's scoring, the Hay/Ison scoring system, and the ison/hay scoring systems.[12-14] The treatment modalities for BV include: metronidazole, tinidazole, clindamycin, probiotics, lactate gel, octenidine hydrochloride and anti-septics.

In this study, we wanted to find out how Pap-smear and vaginal culture measure in their diagnostic accuracy of BV, against each other, using amsel's criteria as the gold standard.

Objective: To compare vaginal culture and pap smear in the diagnosis of bacterial vaginosis

Material &Methods

The study expanded over a period of 5 months, from January 2013- May 2013. During this time we examined 150 reproductive age women (18-35yrs), who were presenting with bad smelling vaginal discharge, itching and epigastric discomfort. Pregnant women and women who were using oral contraceptives, antibiotics or vaginal suppository were excluded from the study. The patients were selected from the out-patient's departments of Obs/Gynae Hayatabad Medical Complex and lady reading hospital Peshawar. Protocol of the study was approved from hospital's ethics review board. All patients had to sign a consent form prior to examination. A tray containing; microscopic slides, vaginal swabs, KOH solution, normal saline, litmus paper strips, container with 95% ethanol, gloves, markers and vaginal speculums was put to bedside alongside with a microscope. All patients were subjected to simultaneous testing for vaginal culture, Amsel's criteria and Pap-staining.

Dry, sterile vaginal speculum was used for examination without application of any antiseptic. pH was evaluated using litmus paper. A high vaginal swab was used to make two slides of the secretions. To one of the slides, saline drops were added covered with cover slip and examined under a microscope then and there. To the second slide, drop of 10% KOH solution was added and the emission of fishy odor was noticed. All those patients who tested positive for presence of 'clue' cells i.e. epithelial cells seen under microscope whose boundaries are studded with bacteria and a whiff test i-e, release of fishy odor as

well as change in color of litmus paper from red to blue, were labeled as having bacterial vaginosis.

For vaginal culture studies, the vaginal swab was rotated to lateral vaginal walls and posterior fornix, and samples were collected. They were put in Amies transport medium. For Pap-staining; slides were prepared after stabilizing the cervix with a Valsellum's forceps. A tongue depressor was inserted and touched upon the ectocervix. It was rotated 360° and the scrapings from both sides were spread onto a microscopic slide, the slides were fixed in 95% ethanol. PAP Staining and Diff Quick staining was done . These slides were evaluated according to Bathesda system. If there was an obvious absence of lactobacilli, a filmy background of coccobacilli and cocoobacili were seen along the edges of cell membranes the smears were evaluated as positive for bacterial vaginosis.

For culture vaginal swabs were inoculated onto plates containing three prepared media namely blood agar, Sabourauds dextrose agar and eosin methylene blue agar. Growths on eosin methylene blue agar and Sabouraud dextrose agar were excluded as being fungal growth. Whereas those specimens that yielded beta-hemolytic colonies on blood agar plates, were labeled as suspected positive for GardnerellaVaginosis. Suspected colonies were selected and identified by Gram stain. Gardnerella Morphotypes: Short bacteria that are Gram negative or Gram variable/lactobacillus: gram positive rods.

Results

A total of 150 patients, who presented with vaginal discharge were examined. Vaginal culture and Pap-test were compared for sensitivity and specificity, positive predictive value and negative predictive values with Amsel's criteria, as the gold standard.

Table -1: Age Distribution

	N	Minimum	Maximum	Mean	Std. Deviation
Age of the Patient	150	18.00	34.00	25.9533	4.57501

The study group of 150 patients had a minimum age of 18 years and a maximum age of 34 years, the mean age being 25.95 years.

BV was diagnosed in 15.3% of patients (n=23) on the Pap-test. It was diagnosed in 18.7% of patients (n=28) on culture. Amsel's criteria diagnosed 16% of patients (n=24) to be positive for BV.

Table 2: Sensitivity & Specificity of Pap smear using AMSEL Criteria as Gold Standard

*Bacterial Vaginosis on AMSEL Criteria * Bacterial Vaginosis on PAP smear Crosstabulation*			Bacterial Vaginosis on PAP smear		Total
			Yes	No	
Bacterial Vaginosis on AMSEL Criteria	Yes	Count	15	9	24
		% within BV on AMSEL Criteria (Sensitivity)	62.5 %	37.5 %	100.0 %
		% within BV on PAP smear (PPV)	65.2 %	7.1%	16.0%
	No	Count	8	118	126
		% within BV on AMSEL Criteria (Specificity)	6.3%	93.7 %	100.0 %
		% within BV on PAP smear (NPV)	34.8 %	92.9 %	84.0%
Total		**Count**	**23**	**127**	**150**

Table 3: Sensitivity & Specificity of Culture Using AMSEL Criteria as Gold Standard

Bacterial Vaginosis on AMSEL Criteria * Bacterial Vaginosis on Culture Cross tabulation			Bacterial Vaginosis on PAP smear		Total
			Yes	No	
Bacterial Vaginosis on AMSEL Criteria	Yes	Count	18	6	24
		% within BV on AMSEL Criteria (sensitivity)	75.0%	25.0%	100.0%
		% within BV on Culture (PPV)	64.3%	4.9%	16.0%
	No	Count	10	116	126
		% within BV on AMSEL Criteria (Specificity)	7.9%	92.1%	100.0%
		% within Bacterial Vaginosis on Culture (NPV)	35.7%	95.1%	84.0%
Total		**Count**	**28**	**122**	**150**

Sensitivity, specificity, positive and negative predictive values of Pap-test came out as 62.5%, 93.7%, 65.2% and 92.9%. Vaginal culture was 75% sensitive, 92.1% specific and positive and negative predictive

values were determined as 64.3% and 95.1% respectively.

Discussion

BV is a very common women's health issue. In addition to the distressing symptoms it causes, due to disturbance in vaginal flora balance, it leads to damaging gynecological and pregnancy complications [16]. This infestation is characterized by a loss of the normally resident hydrogen-peroxide producing lactobacilli that prevent the excessive growth of anaerobes by producing an acidic environment[17]. Among the inconsistently found bacteria are included bacteroids, corynebacterium, lactobacillus, veillonella, ruminococcus and streptococci [18].Women suffering from BV are more likely to be coinfected with Herpes simplex virus type 2, trichomonas vaginalis, Neisseria gonnorhea and HIV[3].

The mean age of patients in our study was 25.95, with a minimum of 18 years and a maximum of 34 years. In another study by S. Akhter et al, in Bangladesh. Majority of patients diagnosed with bacterial vaginosis were within the range of 26 to 35 years [19]. In a study by *A.W Levi et al*, the mean age of participants diagnosed with bacterial vaginosis was 33 years, the ages in the study group ranging from 17-79 years.[12]

In our study, Amsel's criteria detected 24 patients to be positive for BV of the 150 patients. That is, 16 percent of the patients were diagnosed with the disorder. *Luni Y* in Aga Khan University Hospital studied the prevalence of BV in pregnant and non-pregnant women, both symptomatic and asymptomatic, with vaginal discharge and had 16.1 percent of patients diagnosed with BV[20]. This is in agreement with the finding of our study. In another study by *Sami S* and *Baloch S* in Bolan Medical Complex hospital Quetta, BV was diagnosed in 30.7 percent of both symptomatic and asymptomatic patients[21]. Incidence of BV in obstetric patients was very high as observed by *Tariq N* in Holy Family Hospital where it was found out to be 68 percent.[22] The prevalence of BV among non-pregnant women ranges from 15 percent to 30 percent[23]. Our percentage of patients diagnosed with BV according to the gold standard, falls within the same range.

Because of the successful decrease in incidence and mortality of cervical squamous cell carcinoma, Pap tests have been in wide use, since their discovery. One of the secondary uses is the detection of microorganisms. As a result, many clinicians have come to incorporate Pap test in identification of microorganisms, as part of their patient management.[24] For the diagnosis of BV, the sensitivity of Pap test varies from 90 percent to as low as 43 percent. This wide range is the result of use of different morphologic criteria and whether the samples were obtained from the cervix or the vagina[25-26.]

The sensitivity of Pap test came out to be 62.5 percent and the specificity as 93.7 percent. The positive and negative predictive values were calculated to be 65.2 percent and 92.9 percent respectively for the Pap test. Vardar E et al., compared Pap smear and gram stain with Amsel's criteria as the gold standard. Pap test was 93 percent sensitive and 94 percent specific, with a positive predictive value of 86 percent. They determined that gram stain and Pap smear methods gave agreeing results, if amsel's criteria are accepted as the gold standard for diagnosis of BV [10]. Platz Christensen and colleagues determined the sensitivity of Pap smear as 88 percent, specificity as 97%, positive predictive value as 97 % in a study comparing Pap smear and gram stain methods in the diagnosis of BV.[24] Difference here is due to the inconsistency in the criteria used to diagnose BV ie some studies rely only on the presence of clue cells while others focus on criteria specified by Bethesda System, variation in specimen source, studies based on vaginal smears report higher sensitivity for BV than those taking into account cervical/endocervical smears, experience and number of evaluators differ among different studies.

Udyalaxmi et al., while comparing methods for diagnosis of BV, found out that culture was 51% sensitive, and 88.7 % specific, the positive predictive value was 85.5% and negative predictive value was 58%.[27] He concluded that culture was the least sensitive method. C.Tokyol et al., while comparing vaginal culture and Pap test, taking gram-stain as the standard, found that sensitivity of culture was 77.8%, specificity was 97.7%, positive and negative predictive values being 93.3% and 91.4% respectively [26].While in our study, vaginal culture was found to be 75 percent sensitive, 92.1 percent specific, with a positive and negative predictive values of 64.3 percent 95.1 percent respectively. It is noticeable that the results of the two studies are not in a striking contradiction.

Another study concluded that vaginal cultures have excellent sensitivity for the diagnosis of BV, but as the predictive value of a positive Gardnerella vaginalis culture is less than 50%, culture cannot be recommended.[30] Pavani K and Saileela K, while

comparing vaginal culture with Nugent's criteria found out that culture was 42.55% sensitive and 92.99% specific. The positive predictive value was 64.5% and the negative predictive value was 84.39%. They concluded that culture was the least sensitive. method.[3]

Conclusion

Out of these two tests, vaginal culture was labeled as the more sensitive test for the diagnosis of bacterial vaginosis. However, Culture cannot assume the status of being a gold standard test for BV, as most of the microorganisms constituting normal resident vaginal flora cannot be isolated easily in the laboratory.PAP smear has added benefit of its use in detecting cervical carcinoma and is less time consuming. Exclusion of culture and gram stain from the routine examinationmethodwill also decreasetheeconomic cost. It is better tousePapsmearsin routine gynecologic cytological examinations.

Declarations

Undertaking: this paper has not been submitted for a concurrent publication, and has not been published before in any other journal.

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Author's contribution: MI and NS designed the study. MI,ST, SHD and SH collected specimens. MI and SHD performed experiments. MZ SH and MI analyzed the data. MI prepared the manuscript. NS review and approved the final manuscript.

Competing Interest: Authors declare that they have no competing interests

Ethical approval and consent to participate: This study was approved by ethical committee of Khyber Medical University and written informed consent was obtained from all patients

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