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Bacterial vaginosis in Khartoum State, Sudan: Comparison of Gram stain with Pap test procedures

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The prime purpose of this study was to define the reliability of the Pap test in establishing the diagnosis of bacterial vaginosis (BV) as well as ascertaining the trustworthiness of Pap test and Gram stain in the diagnosis of bacterial vaginosis using Amsel's criteria as a gold standard method. Thereby prospective study conducted included 300 females attending to the Department of Gynecology and Obstetrics in our center for the usual follow-up, during a period between December 2013 and August 2014. Every patient had both Pap test and Gram-stained vaginal smear as well. Specifity, sensitivity and predictive values of aforementioned tests were calculated using the obtained results compared to Amsel's criteria as reference values. Adopting Amsel's criteria as a gold standard method, sensitivity and specificity of Pap test were found to be 83.4 and 95.2% respectively, compared to 93.4 and 100% in Gram stain. Whereas the positive and negative predictive values of Pap smear were estimated as 88.2 and 93%, respectively. On the other hand, Gram stain shows positive and negative predictive value of 100 and 97.2% correspondingly. Accounting on data presented in this study, it was concluded that, Pap test is found to be less sensitive for screening of BV comparable to the standard microbiological test results. Notwithstanding, it may be adequately enough owing to its high specificity.

Key words: Bacteria vaginosis, Pap test, Gram stain.

INTRODUCTION

Admittedly, bacterial vaginosis (BV) is the most common cause of vaginal discharge among females of childbearing age, notably, accounting for 40 to 50% of cases (Joesoef and Schmid, 2001). Significantly, the average prevalence of BV varies; the rates are 10 to 35% in females attending the hospital, 10 to 30% in patients visiting obstetric wards

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and 20 to 60% in patients visiting centers of sexually transmitted disease (Georgijevic et al., 2000). Importantly, the diagnostic significance of BV is that it can be associated with many obstetric and gynecologic complications, including puerperal endometritis, preterm labor, chorioamnionitis, preterm premature rupture of membranes, pelvic inflammatory disease, urinary tract infection (UTI), postoperative cellulitis, and precancerous lesions of the cervix (Thomason et al., 1991; Clark et al., 1994).

BV is a common yet poorly understood condition in which the balance of bacteria inside the vagina becomes disrupted and considered as commonly encountered cause of vaginitis. Gardner and Dukes (1955) were the first to report *Haemophilus vaginalis* as a cause of nonspecific vaginitis. Prominently, they defined the term as, “Any woman whose ovarian activity is normal, and who has a gray, homogenous, malodorous vaginal discharge with a pH of 5.0 to 5.5 that yields no Trichomonads is likely to have *H. vaginalis* vaginitis.” Furthermore, it is also known as nonspecific vaginosis/vaginitis, so named by Blackwell and Barlow (1982), or bacterial vaginosis (BV), a term accepted by the International Agency for Research on Cancer. Needless to mention that, Gardner and Dukes (1955) are among the first who reported and described these organisms. Intriguingly, the mechanism underlying the etiology of BV suggested that “BV probably results from infection with complex communities of bacteria that consisted of metabolically interdependent species (Fredricks and Marrazzo, 2006).

The Pap test is commonly used in cytological screening for early detection of cervical intraepithelial lesions of the uterine cervix. Accordingly, the success of cytological screening program for cervical cancer has made the Pap test to be used as a routine test for the early detection of the cervical cancer (Cidem et al., 2004; Catarino et al., 2015). Additionally, it has also been assessed as a diagnostic test for the diagnosis of BV (Hainer and Gibson, 2011; Barouti et al., 2013), nonetheless, the results of these studies are contradictory. Therefore, the aim the current study is to coherently determine the reliability of the cervical smear in establishing the diagnosis of bacterial vaginosis and assessing the characteristics of Pap test in the diagnosis of BV, using the Amsel’s guideline criteria as the gold standard procedure for the diagnosis of BV.

METHODOLOGY

This is a prospective hospital based study which included 300 females referred to the Department of Gynecology and Obstetrics in our center for the usual follow up at Khartoum State, Sudan, during the period between December 2013 and August 2014. A written consent was taken from all participants after explaining the purpose, the sampling process as well as the potential risk. Each patient has had standard Pap test along with Gram-stained vaginal smear.

Reporting of Pap test

Pap test was taken using spatula and endocervical brush, then the samples were spread on slides, and after that immediate fixation was done. This was very useful to avoid air dry artifacts. Afterwards, all samples were then stained using Papanicolaou method. Then, the samples were meticulously assessed and evaluated by double blind expertise cytologist. Then, the samples were rated as satisfactory when transformational zone cells were found, that is, detection of 10 well-preserved endocervical or metaplastic squamous cells isolated or in monolayer sheets. It is worthy noted that specimens’ adequacy were assessed using the Bethesda system guidelines for reporting cervico vaginal smears. Accordingly, if there was a filmy background of small coccobacilli, clue cells, and conspicuous absence of lactobacilli, the smear was reported as positive for bacterial vaginosis. Whereas smears displaying the characteristic patterns of bacterial vaginosis were reported as ‘shift in flora suggestive of bacterial vaginosis’. Afterwards the smears were later re-examined for the presence of BV by two pathologists who were unknowing to the gram stained smear results.

Gram technique and evaluation

The vaginal swab specimens were collected from the posterior fornix. Then were smeared directly on glass slides, and allowed to air dried for a standard Gram stain as the following. After fixation with heat, the smears were stained by crystal violet for 1 min, then rinsed in distilled water, then all the smears were allowed to remain in iodine solution for 1 min, then it was rinsed in tap water, decolorized for 5 s by using acetone, immediately the smears were rinsed in water, then flooded with safranin as counter stain for 45 s, then rinsed in water, all slides were air dried for examination using X100.

Gram-stained slides were ascertained and assessed in compliance with Nugent et al. (1991) guidelines, by a microbiologist who was blinded to the results of Pap test. Each slide was examined under high power field (X1000) for the following morphology: large Gram-positive bacilli (*Lactobacillus*), small Gram-negative/variable bacilli (*Gardnerella* and *Bacterioides*), and curved Gram-negative/variable bacilli (*Mobiluncus*). These criteria were used to develop a 0-10-point scoring system for the diagnosis of BV (Çi dem et al., 2004).

The assessment of the results obtained from Pap and Gram stains were based upon positivity using Amsel’s criteria (Amsel et al., 1983). For a positive diagnosis of BV, the presence of at least three of the following four criteria of Amsel is required: thin, homogeneous gray-white discharge on the vaginal wall, that can be easily sampled; vaginal-discharge pH above 4.5; observation of ‘clue’ cells in fresh vaginal smear; and amine smell similar to that of stale fish, obtained by dripping 10% KOH on the vaginal discharge. Specifity, sensitivity and predictive values of the results obtained from Pap and Gram stains were calculated by using the results obtained according to Amsel’s criteria as reference values.

Ethical clearance

The study was approved by the Reviewers Board, Faculty of Medical Laboratory Science, University of Khartoum. A written consent was taken from all participants after explaining the purpose, the sampling process as well as the potential risk.

Statistical analysis

The obtained data were statistically analyzed using Statistical Package for Social Science (SPSS-v17) used to analyze the

Table 1. Comparisons between Gram stain with Amsel's criteria for the diagnosis of BV.

Gram	Amsel		Total
	Positive	Negative	
Positive	84	0	84
Negative	6	210	216
Total	90	210	300

Table 2. Comparison between Pap test and Amsel's criteria for the diagnosis of BV.

Pap	Amsel		Total
	Positive	Negative	
Positive	75	10	85
Negative	15	200	215
Total	90	210	300

Table 3. Diagnostic value of Gram stain and Pap test in the diagnosis of BV compared with other studies.

Authors	Gram stain		Pap stain	
	Sensitivity	Specificity	Sensitivity	Specificity
Davis et al. (1997)	-	-	55	98
Enver et al. (2002)	97	94	93	94
Çi dem et al. (2004)	-	-	43.1	77.8
This Study	93.4	100	83.4	95.2

applied data and to perform Pearson Chi-square test for statistical significance (P-value). Sensitivity and specificity were calculated.

RESULTS

The present study included 300 females. Their ages ranged between 17 and 65 years, with a mean age of 41 years. Of the 300 Papanicolaou test, importantly, all of them were collected from the transformation zone, therefore, it consisted of endocervical cells as well as squamous cells, notably, among the 300, there were 5 with atypical squamous cells-undetermined significant (ASC-US), 10 smears were diagnosed as LSIL and one was diagnosed as HSIL. It is worthy to note that majority of the females presented with vaginal discharge, looked as a yellow-gray among 78% of cases, whereas 21% show yellow-green color.

Accounting on criteria designed by Amsel et al. (1983) BV was found among 90 (30%) patients. On comparing Pap smear to Gram stain, 75 of our patients were determined as BV-positive by Pap test, compared to 84 positive cases determined by Gram stain (Tables 1 and 2). Sensitivity and specificity of Pap test were found to be 83.4 and 95.2%, respectively, compared to 93.4 and 100% in Gram stain. The positive and negative predictive of Pap smear were calculated as 88.2 and 93%, respectively. Whereas, Gram stain shows positive and negative predictive value of 100 and 97.2%, correspondingly (Table 3).

DISCUSSION

Admittedly, speedily diagnosis of disease offers better treatment thus improving the quality of life. Nearly 50% of females with bacterial vaginosis were considered to be asymptomatic (Georgijevic et al., 2000). To establish the diagnosis of bacterial vaginosis the clinician depends on the clinical signs and symptoms or they referred the patients to do microbiological investigations (Nugent et al., 1991; Mastrobattista et al., 2000). However, BV is often misdiagnosed using clinical criteria because it was found to be subjective and dependent on the performance

of the clinician and existing tools. The use of culture media for *Gardnerella vaginalis* is limited by the test's poor specificity (Davis et al., 1997).

Several techniques have been suggested for the evaluation of preparations for BV by Gram stain. Spiegel et al. (1983) calculated a total of 20 areas in 1000 magnification in which they reported the diagnosis of BV, in correlation with the number of microorganisms present in each large magnification area. Nugent et al. (1991) determined a total score over six as BV, by taking *Lactobacillus* and *Gardnerella* and other microorganisms into account in a total of 20 large magnification areas in 1000 magnification. In the present study, the clue cell scheme of Thomason et al. (1992) has been used.

Platz-Christensen et al. (1995) determined the sensitivity of the Pap smear method as 88%, the specificity as 97% and positive predictive value as 97%, following a study comparing Pap test and Gram stain methods in the diagnosis of BV. These rates were 100, 97, and 94%, respectively for the Gram stain method (Platz-Christensen et al., 1995). In our study, the observed sensitivity and specificity were 83.4 and 95.2%, respectively for Pap test, and 93.4 and 100%, respectively for Gram stain. The positive predictive values were determined as 88.2% for Pap test and 100% for Gram stain. Our results show similarity to that obtains by Platz-Christensen et al. (1995).

Davis et al. (1997), reported that compared to the Gram stain as gold standard method, Pap smear outcomes had a sensitivity of 55%, specificity of 98%, a positive predictive value of 96%, and a negative predictive value of 78%. They reached their hypothesis because of lower rates of sensitivity. Davis et al. (1997) claimed that the fact that Pap test screening is a routine technique and cannot be always evaluated by cytopathologist was the cause of lower rates of sensitivity. But the Pap test reporting criteria given to the cytoscreener, despite a long period of training may explain the different results in their study.

Schnadig et al. (1989) cultured *G. vaginalis* in approximately 90% of the cases that contained clue cells and concluded that they are a high correlation between Pap test and Gram smear in diagnosis of BV. In our recent study, our results shows that Pap test has a specificity of 95.2% and sensitivity of 83.4%, for the diagnosis of BV when compared with Nugent et al. (1991) criteria and 93.4% was the sensitivity and 97.1% specificity of Gram stain.

One of the most significant complications of BV is cervical intraepithelial lesions (Gillet et al., 2012; Biswal et al., 2014; Mitra et al., 2016). It is postulated that bacterial vaginosis could play a very important role in the development of cervical cancer; this may be attributed to biochemical changes in vaginal secretions of women with BV which include production of metabolic by-products, such as propionate and butyrate, capable of damaging epithelial cells. In addition, the BV-associated anaerobes release volatile amines (especially putrescine, trimethylamine and cadaverine), responsible for the characteristic fishy malodour. Amines appear in the vaginal environment after conversion of amino acids produced by abundance of anaerobes, and form in combination with nitrites (produced by nitrate reducing bacteria) nitrosamines. These carcinogenic compounds are capable of forming DNA adducts and consequently mutation (Gillet et al., 2012). In our study, only one out of two females had cervical intraepithelial neoplasia grade III and five out of ten had low grade squamous intraepithelial lesion and all of the six patients were positive for bacterial vaginosis.

Although, Pap test are a simple technique for screening cervical cancer and its sensitivity is low when compared with the microbiological tests for screening of bacterial vaginosis. Nevertheless, due to high specificity of the Pap test, it may be adequate diagnostic criteria when it is positive.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Amsel R, Totten PA, Spiegel CA, Chen KC, Eschenbach D, Holmes KK (1983). Nonspecific vaginitis. Diagnostic criteria and microbial and epidemiologic associations. Am. J. Med. 74(1):14-22.

Barouti E, Farzaneh F, Sene AA, Tajik Z, Jafari B (2013). The Pathogenic Microorganisms in Papanicolaou Vaginal Smears and Correlation with Inflammation. J. Fam. Reprod. Health 7(1):23-27.

Biswal B, Singh K, Ismail M, Jalal M, Safruddin E (2014). Current Concept of Bacterial Vaginosis in Cervical Cancer. J. Clin. Gynecol. Obstet. 3 (1):1-7.

Blackwell A, Barlow D (1982). Clinic diagnosis of anaerobic vaginosis (non-specific vaginitis). A practical guide. Br. J. Vener. Dis. 58:387-393.

Catarino R, Petignat P, Dongui G, Vassilakos P (2015). Cervical cancer screening in developing countries at a crossroad: Emerging technologies and policy choices. World J. Clin. Oncol. 6(6):281-290.

Çi dem T, Orhan C, Arif S, Mustafa A, Fatma. 2004. Bacterial vaginosis: comparison of Pap smear and microbiological test results. Mod. Pathol. 17:857-860

Clark P, Kurtzer T, Duff P (1994). Role of bacterial vaginosis in peripartum infections. Infect. Dis. Obstet. Gynecol. 2:179-183.

Davis JD, Connor EE, Clark P, Wilkinson EJ, Duff P (1997). Correlation between cervical cytologic results and Gram stain as diagnostic tests for bacterial vaginosis. Am. J. Obstet. Gynecol. 177:532-535.

Enver V, Izzet M, Murat I, Ozgül O, Tasli F, Postaci H (2002). Comparison of Gram stain and Pap smear procedures in the diagnosis of bacterial vaginosis. Infect. Dis. Obstet. Gynecol. 10(4):203-207.

Fredricks D, Marrazzo J (2006). Bacteria associated with bacterial vaginosis. N. Eng. J. Med. 354:202-203.

Gardner HL, Dukes CD (1955). Haemophilus vaginalis vaginitis. Am. J. Obstet. Gynecol. 69:962-976.

Georgijevic A, Cjukic-Ivancevic S, Bujko M (2000). Bacterial vaginosis. Epidemiology and risk factors. Srp Arh Celok Lek. 128:29-33.

Gillet E, Meys JFA, Verstraelen H, Verhelst R, De Sutter P, Temmerman M, Broeck DV (2012). Association between Bacterial Vaginosis and Cervical Intraepithelial Neoplasia: Systematic Review and Meta-Analysis. PLoS One 7(10):e45201.

Hainer BL, Gibson MV. 2011. Vaginitis: Diagnosis and Treatment. Am. Fam. Physician 83(7):807-815.
Joesoef M, Schmid G (2001). Bacterial vaginosis. In Clinical evidence, BMJ Publishing Group, London. P 887.
Mastrobattista J, Bishop K, Newton E (2000). Wet smear compared with Gram stain diagnosis of bacterial vaginosis in asymptomatic pregnant women. Obstet. Gynecol. 96:504-506.
Mitra A, MacIntyre DA, Marchesi JR, Lee YS, Bennett PR, Kyrgiou M (2016). The vaginal microbiota, human papillomavirus infection and cervical intraepithelial neoplasia: what do we know and where are we going next? Microbiome 4(1):58.
Nugent R, Krohn M, Hillier S (1991). Reliability of diagnosing bacterial vaginosis is improved by a standardized method of Gram stain interpretation. J. Clin. Microbiol. 29:297-301.
Platz-Christensen J, Larsson P, Sundstrom E, Wiqvist N (1995). Detection of bacterial vaginosis in wet mount, Papanicolaou-stained vaginal smears and in Gram-stained smears. Acta Obstet. Gynecol. Scand. 74:67-70.
Schnadig VJ, Davie KD, Shafer SK, Yandell RB, Islam MZ, Hannigan EV (1989). The cytologist and bacteriosis of the vaginal–ectocervical area: clues, commas and confusion. Acta Cytol. 33:287-297.
Spiegel C, Amsel R, Holmes K (1983). Diagnosis of bacterial vaginosis by direct Gram stain of vaginal fluid. J. Clin. Microbiol. 18:170-177.
Thomason JL, Anderson RJ, Gelbart SM, Osypowski PJ, Scaglione NJ, El Tabbakh G, James JA (1992). Simplified Gram stain interpretive method for diagnosis bacterial vaginosis. Am. J. Obstet. Gynecol. 167:16-19.
Thomason JL, Gelbart SM, Scaglione NJ (1991). Bacterial vaginosis: current review with indications for asymptomatic therapy. Am. J. Obstet. Gynecol. 165:1210-1217.