

Comparison of the Use of Papanicolaou-stained Cervical Cytological Smears with Gram-stained Vaginal Smears for the Diagnosis of Bacterial Vaginosis Among Out-Patient Pregnant Patients

CLAIRE LIZ I. BOMBASE, MD AND ANALYN T. FUENTES-FALLARME, MD, FPOGS*

Department of Obstetrics and Gynecology, Philippine General Hospital, University of the Philippines-Manila

ABSTRACT

Background: Bacterial vaginosis is the most common vaginal infection among reproductive age women. It has been associated with preterm labor and emerged as a formidable disease entity associated with catastrophic sequelae especially in pregnant patients. Papanicolaou smear is the most successful screening test for cervical carcinoma in the history of medicine. Albeit being used by some clinicians as screening tool for bacterial vaginosis, it was not well established.

Objective: To determine the diagnostic accuracy of papanicolaou smear in making the diagnosis of bacterial vaginosis in pregnant patients with the vaginal gram stain used as diagnostic standard.

Methodology: A total of 321 pregnant patients who consulted for prenatal care at the outpatient department of obstetrics and gynecology of the tertiary training hospital between November 2013 to June 2014 were included in the study. Each patient had gram-stained vaginal smear and standard pap smear done consecutively. The sensitivity, specificity, positive predictive value, negative predictive value and the likelihood ratios of pap smear were determined.

Results: The sensitivity of pap smear in determining the presence of bacterial vaginosis is 70.5%, its specificity of 93.6%, positive predictive value of 80.5%, negative predictive value of 89.3%. Likelihood ratio of a positive result is 10.9 and 0.3 for a negative result with diagnostic accuracy of 87.23%.

Conclusion: These results support the reliability of the pap smear in the diagnosis of bacterial vaginosis in asymptomatic pregnant patients. Since pap smear and gram stain have close diagnostic accuracy, duplication of the test could reasonably be avoided in most patients.

Keywords: bacterial vaginosis, pap smear, gram stain

INTRODUCTION

Outmost care of the pregnant women is of paramount importance to obstetricians. Antenatal care is designed for the holistic care of the mother and unborn child to prevent pregnancy complications. It has been recognized as a cornerstone of health care system for pregnant women since the beginning of the 20th century. It is important to the health of the unborn baby hence at the initial prenatal check-up, obstetricians request a number to diagnostic procedures to identify diseases that may complicate their pregnancy.

Bacterial vaginosis, a seemingly benign condition, is often associated with various devastating sequelae in

pregnancy. It is the leading cause of vaginitis and affects over 3 million women annually, including about 60% of the patients seen at sexually transmitted disease (STD) clinics. It is one of the most common genital tract infections in pregnant women, affecting approximately 12-22% of women.² However, over 50% of women with bacterial vaginosis do not show symptoms. Vaginal discharge is common in pregnancy and may be physiologic. One of the differential diagnoses in pregnant patient with pelvic examination findings of whitish vaginal discharge is bacterial vaginosis. More often it is mistaken as the physiologic leukorrhea of pregnancy since most patients with bacterial vaginosis do not show symptoms.

Untreated bacterial vaginosis in non-pregnant women typically remains a local inflammation, producing symptoms in the vagina only. Bacterial vaginosis in pregnant women was once regarded as benign nuisance as well; however, at least nine recent clinical studies have shown that bacterial vaginosis during pregnancy is associated

* First Place, Philippine Obstetrical and Gynecological Society (POGS) 2014 Residents' Research Paper Contest, October 24, 2014, POGS Assembly Hall, Quezon City

with preterm labor, preterm delivery and premature rupture of membranes.² It is associated with two-three times increased risk of preterm delivery compared to those without infection. Furthermore, it is associated with increased risk of intrapartum and postpartum infections.

Current scientific evidence suggests the association between bacterial vaginosis and adverse pregnancy outcomes is actually a cause and effect link as shown in studies that reduction in bacterial vaginosis leads to reduction in adverse pregnancy outcomes.² In the most recent randomized controlled trial in UK, treatment of asymptomatic women with bacterial vaginosis or intermediate flora detected on gram stain produced a five-fold reduction in late miscarriage and two and a half fold reduction in spontaneous preterm birth.³ In the recent Cochrane review done in 2012, 21 randomized trials involving 7847 women have been identified. Antibiotic therapy was shown to be effective at eradicating bacterial vaginosis during pregnancy with risk ratio (RR) of 0.42 and reduced the risk of late miscarriage, RR of 0.20 but treatment before 20 weeks age of gestation did not reduce the risk of preterm birth before 37 weeks RR of 0.88 nor the risk of premature prelabor rupture of membranes RR 0.74. However, it was noted in two studies that in women with abnormal vaginal flora (indeterminate flora or bacterial vaginosis), treatment may reduce the risk of preterm birth before 37 weeks by 47%, RR 0.53.⁴

Currently, there is no consensus as to whether to screen for or treat bacterial vaginosis in general population. In SOGC clinical practice guidelines, asymptomatic women without risk factors for preterm birth should not undergo routine screening or treatment for bacterial vaginosis but those with increased risk for preterm birth may benefit from routine screening and treatment of this condition.⁵

In CDC sexually transmitted disease treatment guidelines of 2010, bacterial vaginosis can be diagnosed by the use of clinical criteria (Amsel's criteria) or Gram stain, the gold standard for diagnosis. Cervical Papanicolaou tests have limited clinical utility in diagnosis of bacterial vaginosis but its potential as a diagnostic test has been explored by several authors.

The pap smear has been widely used since 1940s and is the most successful screening test for carcinoma in the history of medicine.⁷ Papanicolaou smear is recommended to pregnant patients regardless of the duration of pregnancy at her first prenatal visit as part of her initial evaluation. Its main benefit is early detection of preneoplastic lesions of the cervix, however, several infections such as bacterial vaginosis is also reported on its findings. Pap smear ability to diagnose bacterial vaginosis is not well established. In a pregnant patients mostly presenting with vaginal discharge which may be physiologic in addition to unawareness of symptoms of

bacterial vaginosis, clinical diagnosis of bacterial vaginosis is challenging. Since routine screening for bacterial vaginosis is not recommended, will a pap smear finding of bacterial vaginosis sensitive or specific in diagnosing the condition?

Several studies were done in the past evaluating the diagnostic accuracy of pap smear in detecting bacterial vaginosis using gram stain as a gold standard but it yielded varied results. In 1999, Lamont et al revealed the pap smear's sensitivity of 80.7%-87% and specificity of 90.7-97% in their study done in pregnant patients. Good diagnostic accuracy of pap smear in detecting bacterial vaginosis was also documented by Lipana last 2006 in her study on gynecologic and obstetric patients in the same institution. Pap smear has sensitivity of 94.1% and specificity of 88.2%. The most recent study by Raina et al last 2013 on gynecologic patients showed a lower sensitivity of 61% but still high specificity of 97.6%. To date, no published local study was done evaluating the diagnostic accuracy of pap smear in detecting bacterial vaginosis among pregnant women.

Given the varying results of the studies determining the diagnostic value of pap smear in the detection of bacterial vaginosis, this study aims to evaluate the diagnostic accuracy of pap smear in detecting bacterial vaginosis in pregnant patients consulting in our institution. In this age when cost efficiency seems so important especially in pregnant patients with low socioeconomic status consulting in a tertiary government hospital, the contribution of the pap smear in the initial evaluation of pregnant patients should be seriously considered. For a condition with such a wide spectrum of catastrophic sequelae especially in pregnant patients who are most of the time asymptomatic or unaware of its symptoms, the clinical significance of bacterial vaginosis detected in pap smear will be explored.

OBJECTIVES

To determine the diagnostic accuracy of papanicolaou smears in detection of bacterial vaginosis among pregnant patients seeking antenatal care in the outpatient department of a tertiary training hospital by comparing the sensitivity and specificity of papanicolaou-stained cervical cytology smears with a standardized method of interpreting gram-stained vaginal smears

Specific Objectives:

1. To determine the demographic profile of pregnant patients seeking antenatal care in the outpatient department of a tertiary training hospital
2. To determine the prevalence of bacterial vaginosis

among pregnant patients seeking antenatal care in the tertiary training hospital

3. To determine the sensitivity, specificity, positive predictive value, negative predictive value and the likelihood ratios of pap smear in diagnosing bacterial vaginosis compared with gram stain as the gold standard

METHODOLOGY

This study was reviewed and approved by the institution's technical and ethical review board.

Study design

We performed a descriptive, prospective, blinded study involving 321 pregnant patients who consulted for prenatal care at the outpatient department of obstetrics and gynecology of the tertiary training hospital between November 2013 to June 2014. Each patient had gram-stained vaginal smear and standard pap smear done consecutively.

Patient population:

The patient population in the study included pregnant women who consulted for prenatal care at the outpatient department who satisfied the following criteria:

Inclusion Criteria:

1. Age between 18-35 years
2. On their 12-34 weeks age of gestation
3. Absence of vaginal bleeding
4. No coitus within the last 3 days prior to consult
5. Did not receive any treatment for Cervicovaginal infections within the last two weeks prior to consult
6. Did not use vaginal spermicides and creams, vaginal douche, tampons within the last 24 hours prior to consult
7. Patients with informed consent

Exclusion Criteria:

1. Patients with concomitant genital tract infections

Sample size

Using the sample size formula for sensitivity and specificity studies, the minimum sample size requirement of 321 patients were needed to achieve the precision of 5% for specificity given that the expected specificity of pap smear is 88.2% (Lipana, 2006) and the prevalence of bacterial vaginosis among pregnant women is 50% (Nelson et al, 2002).

The sample size formula for the estimation of specificity:

$n = [z^2 * P(1-P)] / \Delta^2$ where P is sensitivity/specificity and Δ is precision.

Non probability sampling was employed in patients' selection at the outpatient department.

Description of the study procedure

Pregnant patients who fulfilled the criteria were included in the study. The principal investigator or the available residents explained the details of the study to the patients. Informed consent was obtained from patients who accepted and agreed to be a part of the investigation.

A clinical evaluation sheet noting the patient's name, age, gravidity/parity, medical record number, date of exam, gestational age and pelvic examination findings was filled out by obstetrics and gynecology residents providing patient care. Vaginal inspections were carried out using a sterile speculum without applying antiseptic cleansing liquids and the characteristics of the vaginal discharge were reported. Presence of leukorrhea or other vaginal discharge was noted. A sample of vaginal discharge for gram stain and standard papanicolaou smear of the cervix were obtained consecutively by residents assigned at the outpatient department to all patients included in the study. Vaginal smears were collected from the posterior fornix, also with cotton tip applicator. The vaginal swabs specimens were smeared directly on the glass slide and air dried for the standard gram stain. Pap smear were done in routine manner using cotton tip applicator, fixed with 95% alcohol for fifteen minutes and stained by the papanicolaou method. All papanicolaou smears were submitted to the cytology laboratory of the said tertiary hospital for examination. Pap smear were evaluated using Bethesda system guidelines for specimen adequacy and general categorization. The smear will be reported as "positive for shift in vaginal flora suggestive of bacterial vaginosis" with a semi-quantitative scoring 0f + to +++ based on the following characteristics: 1. Clear evidence of a filmy background of small coccobacilli; 2. Presence of individual squamous cells with a layer of coccobacilli along the margins of cell membranes and 3. Conspicuous absence of lactobacilli. On the other hand, one cytotechnician/ cytopathologist blinded to the clinical findings and results of pap smear performed the gram stain interpretation to ensure the reproducibility of the gram stain finding. Gram stained slide were evaluated according to Nugent's criteria to characterize 3 grades of vaginal flora and diagnose bacterial vaginosis. Each slide was examined under oil immersion objective for the following morphotypes: large gram-positive rods (lactobacillus morphotypes), small gram-negative/variable rods (Gardnerella and Bacteroides morphotypes) and curved gram negative/variable rods (Mobiluncus

morphotypes). The number of each bacterial morphologic type from 1 to 4+ in a typical oil immersion field were counted and average obtained for scoring using a 0-10 point scoring system based on Nugent's criteria for the diagnosis of bacterial vaginosis (Table 1). The scores of all three bacterial morphologic type categories were added and final scores were obtained. Final scores of 7-10 are diagnostic of bacterial vaginosis, 4-6 is indeterminate and 0-3 was normal smear result. For the purposes of this study, patients with indeterminate scores were considered to be negative.

The results of the gram stains and pap smear were compared and analyzed with gram stain used as diagnostic standard.

Data Analysis

All information collated from the subjects were recorded and analyzed utilizing statistical software "Open Epi". Descriptive statistics using point and interval estimates were performed on patients' profile. Diagnostic characteristics that included sensitivity, specificity, positive predictive value, negative predictive value and likelihood ratios were calculated using standard definitions.

RESULTS

A total of 321 pregnant patients who consulted at the outpatient department were included in the study. The mean age of the subjects was 26.5 years (range 18-35). Most of the pregnant patients, 31% were 22-25 years old, while the least number, 14% were in 18-21 year-old group. The mean gravidity of the patients was 2.4 (range 1-7) majority of them, 66% were multigravid. The mean parity was 1.2 (range 0-5), most of them were nullipara, 37%. Two thirds of the pregnant patients were single. The average age of gestation of the subjects on their first prenatal check up is 24.4 weeks (range 12-35 weeks). Majority of them were between 20-27 weeks.

On pelvic examination, 261 of 321 pregnant patients had leukorrhea. Majority of them, 58 % were assessed to have mild leukorrhea. Bacterial vaginosis was clinically diagnosed in 21 cases of those assessed to have moderate to severe leukorrhea. Of the 21 cases of clinically diagnosed bacterial vaginosis, 9 yielded positive results in gram stain, 10 in the pap smear while only 7 (33%) patients were positive for bacterial vaginosis in both pap smear and gram stain. Clinical assessment of bacterial vaginosis by the residents gave a false positive rate of 57% using gram stain as gold standard for diagnosis.

The gram stain using Nugent's criteria was able to detect 88 cases of bacterial vaginosis out of the 321 patients included in the study, giving a prevalence of 28%.

Table 1: Scoring system for gram stained vaginal smears

Scores	Lactobacillus morphotypes	Gardnerella and Bacteroides spp. morphotypes	Curved gram-variable rods
0	4+	0	0
1	3+	1+	1+ or 2+
2	2+	2+	3+ or 4+
3	1+	3+	
4	0	4+	

Table 2: Classification of Scores

Score	Group
0-3	Normal
4-6	Indeterminate
7-10	Bacterial vaginosis

There were 4 patients with indeterminate scores and were counted as negative for bacterial vaginosis. On the otherhand, out of 321 pap smears performed, 77 had shift in flora suggestive of bacterial vaginosis, rate of 24%.

The prevalence of bacterial vaginosis increases as the age group increases with the highest number of positive results in gram stain among 31-35 year-old age group with 8.1% prevalence. Prevalence of bacterial vaginosis in age of gestation at the time of initial consult showed that more than 1/3 of those positive in gram stain were in their early third trimester, 29-34 weeks (10%) and early second trimester, 12-19 weeks (9.7%).

Pap smear findings were compared to the results of the gram stain, the gold standard, concomitantly obtained from the patient. The pap smear finding of bacterial vaginosis was considered "true positive" if it was confirmed positive in the gram stain specimen using Nugent's criteria. A "true negative" meant no bacterial vaginosis identified in both pap smear and gram stain specimen. A "false positive" result meant that bacterial vaginosis was identified on pap smear but not confirmed on gram stain. A "false negative" result meant non-identification of bacterial vaginosis on pap smear but with infection confirmed on gram stain.

Pap smear showed bacterial vaginosis in 62 out of 88 patients who tested positive on gram stain. This gives a sensitivity of 70.45%. On the other hand, pap smear showed negative results for bacterial vaginosis in 218 out of 233 patients with negative results on the gram stain. This gives a specificity of 93.56%. 15 patients who had bacterial vaginosis on pap smear had negative results on gram stain giving a false positive rate of 4.67 % while 26 patients who tested positive on gram stain had negative

pap smear result hence a false negative rate of 8.10 %. The positive predictive value was 80.52% while the negative predictive value was 89.43%. The likelihood ratio for a positive test 10.94 while likelihood ratio of the negative test is 0.32. The overall diagnostic accuracy is 87.23%.

DISCUSSION

Bacterial vaginosis (BV) is one of the conditions often link to the causes of preterm labor. It is the most common lower genital tract disorder among women of reproductive age and also the most prevalent cause of vaginitis presenting as vaginal discharge and malodour.⁷ Bacterial vaginosis was first described by Gardner and Dukes.¹ They regarded *Gardnerella vaginalis* as the etiologic agent of bacterial vaginosis, but this concept has been changed. Bacterial vaginosis is a polymicrobial clinical syndrome resulting from the replacement of the normal hydrogen peroxide producing *Lactobacillus sp* in the vagina with high concentrations of anaerobic bacteria (e.g. *Prevotella sp*, *Mobiluncus sp.*), *G. vaginalis*, *Ureaplasma*, *Mycoplasma*, *Bacteroides* and numerous fastidious or uncultivated anaerobes.⁶ The prevalence of BV among nonpregnant women ranges from 15 percent to 30 percent; up to 50 percent of pregnant women have been found to have BV.¹¹ Other studies showed prevalence ranging from 6% to 32% in pregnant women which is the same as in non pregnant populations with the overall prevalence of 14%.⁵ The prevalence of bacterial vaginosis varies in different populations as shown in various studies. Tokyol et al in 2004 reported prevalence of bacterial vaginosis based on their study to be 29.4%. In the study done in the same institution by Lipana last 2006, prevalence of bacterial vaginosis was 16.7%. In the study of Lata et al in 2010, it was reported to have a prevalence of 20.5% while that of Walker et al in 2011 in the study of Australian women reported its prevalence to be 11.8% and incidence of 8.8 %. Mengistie Z et al in the study done among 252 pregnant women in Ehtiopia last 2013, bacterial vaginosis has a prevalence of 19.4% based on gram stain using Nugent's criteria. In the current study, the rate of those who tested positive for bacterial vaginosis based on gram stain was 28% while those who tested positive in pap smear was 24%. Such result was comparable with that of the study of Tolyol et al but is higher than the previous reported prevalence in the same institution. The wide diversity in the reported prevalence in various studies may be a result of different populations studied, different sample size and different objective criteria used to diagnose the condition. Despite the wide variation in the prevalence and incidence of this clinical entity, it is important to keep in mind that over 50% of women with bacterial vaginosis are asymptomatic and remain unreported and untreated.⁶

The importance of this endeavour is supported by numerous studies showing that bacterial vaginosis has dire consequences especially in pregnant patients. Bacterial vaginosis has been associated with a significant number of obstetric complications, such as preterm labour and delivery, preterm premature rupture of membranes, spontaneous abortion, low birth weight baby, chorioamnionitis, postpartum endometritis and post-

Results of Pap smear and Gram stain

		Gram stain		Total
		Positive	Negative	
Pap Smear	Positive	62	15	77
	Negative	26	218	244
		88	233	321

Caesarean delivery wound infections.⁵ Bacterial vaginosis in pregnancy does not only affect the mother but also has tremendous impact on the long term health condition of the unborn fetus. The presence of bacterial vaginosis at a particular gestational age may be a factor in the subsequent development of pregnancy complications and the risk for the disease may change based on its positivity during different stages of gestation. Studies have shown a two-fold to three-fold increase in preterm delivery with those diagnosed with BV in early second trimester. In the current study, majority of the patients who consulted for their initial prenatal check up at the outpatient department are in their 2nd trimester with the mean age of gestation of 24.4 weeks. More than one third of the reported prevalence in the current study is at 12-19 weeks of gestation. A meta-analysis reviewing the role of BV and the risk of preterm delivery reported a summary odds ratio of 1.6, indicating a 60% increased risk of preterm delivery among pregnant women with BV.¹¹ These complications may be related to increased potential for other vaginal pathogens to gain access to the upper genital tract, presence of enzymes that reduce the ability of leukocytes to reduce infection and an increased level of endotoxins stimulating cytokine and prostaglandin production that increases uterine contractions.¹¹ In a study done by Mass et al, higher incidence of chorioamnionitis and preterm delivery were found in patients with coccobacilli and clue cells on their initial papsmear.

Bacterial vaginosis is a syndrome that can be diagnosed both clinically and microbiologically. BV can be diagnosed by the use of clinical criteria, Amsel's Diagnostic Criteria or Gram stain, which is considered the gold standard.⁶ Bacterial vaginosis is diagnosed using Amsel's

criteria in the presence of three of the following: 1. homogeneous, thin, white discharge that smoothly coats the vaginal walls; 2. presence of clue cells (greater than 20%) on microscopic examination; 3. pH of vaginal fluid >4.5; or 4. a fishy odor of vaginal discharge before or after addition of 10% KOH (i.e., the whiff test). Clinical diagnosis of bacterial vaginosis during pregnancy can be difficult due to physiologic changes occurring during pregnancy. An example of this is the leukorrhea of pregnancy which is a milky discharge with mild odor or no odor at all and is primarily composed of old cells and natural bacterial flora. It shows up increasingly during pregnancy due to elevated levels of estrogen and blood flow.¹⁶ This can be mistaken to the homogenous whitish discharge of bacterial vaginosis or conversely bacterial vaginosis may just be mistaken as physiologic leukorrhea of pregnancy. In this study, 81% of the pregnant patients had leukorrhea as seen on the speculum examination. Since majority, 58% were reported to have mild leukorrhea, bacterial vaginosis was clinically diagnosed in only 7% of the pregnant patients compared to 28% of those detected on gram stain. These patients clinically diagnosed with bacterial vaginosis had moderate to severe leukorrhea on speculum exam.

On the other hand, gram stain of vaginal fluid is the most widely used and evaluated microbiologic diagnostic method for bacterial vaginosis using Nugent's criteria, most commonly used system in its evaluation.⁵ Gram stain-based diagnosis is reliable, reproducible and has the applicability for screening large populations. Gram stain is considered the gold standard laboratory method for diagnosing BV and is used to determine the relative concentration of lactobacilli (i.e. long Gram-positive rods), Gram-negative and Gram-variable rods and cocci (i.e. *G. vaginalis*, *Prevotella*, *Porphyromonas*, and peptostreptococci), and curved Gram-negative rods (i.e. *Mobiluncus*) characteristic of BV.⁶ Gram stained vaginal smear can be evaluated with the Hayson/Ison criteria or Nugent's criteria.³ Hayson/Ison criteria categorizes vaginal flora as Grade 1 (Normal), Grade 2 (indeterminate) and Grade 3 (bacterial vaginosis) whether lactobacillus morphotypes predominate or Gardnerella or Mobiluncus morphotypes predominate. Nugent's scoring system which is the standard for research is similar to Hayson/Ison criteria. It also categorizes vaginal flora as normal, intermediate or with bacterial vaginosis but uses scoring system of 0-10. A score of more than 7 is indicative of bacterial vaginosis and the score of less than 3 is negative for bacterial vaginosis. Women with intermediate flora have increased risk of acquiring gonorrhoea, Chlamydia, trichomoniasis and bacterial vaginosis when compared with women with normal flora.⁹ In a study of Nelson et al, gram stain showed a high sensitivity, 89% and high specificity, 83% when compared to Amsel's criteria.

Despite the gram stain's proven utility in the diagnosis of bacterial vaginosis, there is currently no consensus as to whether to screen for or treat bacterial vaginosis in general pregnant population in order to prevent adverse outcomes, such as preterm birth.⁵ On the other hand, the obstetric patient should receive a Pap smear at her first prenatal visit regardless of the duration of pregnancy as part of her initial evaluation to establish the presence or absence of squamous intraepithelial lesions, sexually transmitted diseases and bacterial vaginosis, all of which represent a risk to fetus and mother.⁷ Hence, the pap smear, if proven accurate, offers a cost effective alternative for identification of patients with bacterial vaginosis.

Pap smear, albeit being used by some clinicians as screening tool for bacterial vaginosis, is not well established. The papanicolaou smear is the most successful screening test for carcinoma in the history of medicine. Its main benefit is the early detection of preneoplastic lesions however several infectious agents that manifest with specific cytologic features may also be diagnosed.⁷ It is a routine screening test used for the detection of cervical abnormalities and precancerous dysplastic changes of the uterine cervix. It can also detect certain viral, bacterial, and fungal infections of the cervix and vagina.¹⁷ Using Bethesda system, pap smears with bacterial vaginosis are typically reported as "predominance of coccobacilli consistent with the shift in the vaginal flora". The diagnosis of bacterial vaginosis is based on the following parameters: presence of a filmy background of small coccobacilli, existence of individual squamous cells with a layer of coccobacilli along the margins or the cell membranes and conspicuous absence of lactobacilli.¹⁸ It is this particular cytopathologic diagnosis that has prompted investigators to determine the pap's smears utility in diagnosing bacterial vaginosis.

Since gram stain has been established as reliable diagnostic tool for bacterial vaginosis, it was used as gold standard in this study in determining the accuracy of pap smear in detecting this clinical condition. Based on the current study, the sensitivity of pap smear in the diagnosis of bacterial vaginosis is 70.45%. This is lower than the sensitivity reported by Lamont et al in 1999 which is done on women in their early pregnancy. The reported sensitivity of pap smear is 80.7 % when interpreted by several cytotechnicians and 87% when interpreted by single cytotechnician. Similarly, the sensitivity in the current study is also lower than the reported sensitivity of Lipana in 2006 done in the same institution on both obstetric and gynecologic patients. The sensitivity was 94.1%, the pap smear was interpreted by single pathologist. However, in the most recent study on diagnostic accuracy of pap smear done by Raina et al in 2013 in gynecologic patients, its sensitivity is 61% which is lower than the sensitivity in this study. An important factor contributing

to varied results is the interobserver variability. Studies cited utilizing several cytopathologists in interpreting pap smear result, including the current study reported lower sensitivity compared to those utilizing single cytopathologist. Furthermore, the expertise and deftness of the cytopathologists in the interpretation of the smears using Bethesda system contributed to inconsistency of results. Lastly, the population studied whether pregnant patients, gynecologic patients or both may also be a factor. On the studies mentioned, only Lamont et al studied pregnant patients as with the current study. According to Michael, interpretation of pap smear obtained from pregnant patients can be difficult due to diagnostic pitfalls that result from physiologic changes that occur during pregnancy. Inflammation by itself on Pap smear is not necessarily indicative of infection and an inflammation is commonly seen on pap smears of pregnant women.⁷ This might explain the higher false negative rate and lower sensitivity of the current study compared to the sensitivity done in the same institution by Lipana on gynecologic and obstetric patients.

Another limiting variable for the discrepancy of pap smear reading with that of the gram stain is the variation in the specimen source. Specimens for pap smear were obtained from the cervix and not the vagina. Studies evaluating vaginal smears report higher sensitivity for bacterial vaginosis than those evaluating cervical/endocervical smears.⁷ Since bacterial vaginosis is a vaginal infection, samples should be obtained from the affected area to achieve a more reliable result. However, since the study is on the diagnostic accuracy of pap smear in detection of bacterial vaginosis, routine cytopathologic conditions for collection and interpretation should be applied.

In terms of specificity, the findings in the current study were comparable with previous studies. The specificity in this study was 93.6%, Lamont et al reported 90.7% with several cytopathologist, 97% with single cytopathologist, Lipana, 88.2% and Raina et al, 97.6%. Notable in the findings in the previous study, majority reported a higher specificity of pap smear in detecting bacterial vaginosis than its sensitivity. This holds true in the current study. Hence, pap smear is a better confirmatory test than screening test.

The positive predictive value obtained in the current study was 80.5 % while the negative predictive value was 89.3%. It is comparable with the findings of Raina et al which gave a value of 85.7% and 91.6% respectively. Positive and negative predictive values describe the patient's probability of having the disease once the results of his or her test are known but they are determined by the prevalence of disease condition for which the test is used.¹⁹ They vary with changing prevalence of the

disease hence have limited clinical usefulness in bacterial vaginosis, a disease with diverse prevalence. Conversely, likelihood ratios are independent of disease prevalence.²⁰ They are useful and practical way of expressing the power of diagnostic tests in increasing or decreasing the likelihood of disease. In the current study, the likelihood ratio of a positive test is 10.9 which means that the likelihood of the patient having bacterial vaginosis has increased by approximately 11 fold given the positive pap smear result. The high specificity and positive likelihood ratio of pap smear in the diagnosis of bacterial vaginosis, a positive diagnosis is of definite diagnostic value.

CONCLUSION

This research endeavored to evaluate the diagnostic accuracy of papanicolaou smears in detection of bacterial vaginosis in pregnant patients by comparing it to gram stain which is the gold standard. The pregnant patients included in the study have mean age of 26.5 years, mostly unmarried multigravid on their 20-27 weeks age of gestation. The prevalence of bacterial vaginosis was 28% based on the gram stain with the highest prevalence noted in 31-35 years old and at 29-34 weeks age of gestation.

Based on the results of the study, the sensitivity of pap smear in determining the presence of bacterial vaginosis is 70.5%, its specificity is 93.6%, positive predictive value of 80.5%, negative predictive value of 89.3%. Likelihood ratio of a positive result is 10.9 and 0.3 for a negative result with diagnostic accuracy of 87.23%.

The results of this study support the reliability of the pap smear in the diagnosis of bacterial vaginosis in asymptomatic pregnant patients. Since pap smear and gram stain have close diagnostic accuracy, duplication of the test could reasonably avoided in most patients.

LIMITATIONS

Different residents from different year levels did the pelvic examination and collection of vaginal smears and pap smears. Furthermore, the site of collection of gram stained smears which is in the vaginal canal is different from the site of collection of pap smear which is in the endocervix and ectocervix. These may contribute to the evaluation of diagnostic accuracy of pap smear using gram stain as gold standard.

RECOMMENDATION

The authors of this study recommends that a further study be done on asymptomatic patients with a positive pap smear result determining how many of them will have pregnancy complications to attempt in making

recommendations regarding prudence of treating patients with bacterial vaginosis on pap smear.

Furthermore, addition of vaginal swab to the standard

pap smear as was previously done, may increase the sensitivity of pap smear in detecting bacterial vaginosis.

REFERENCES

1. Tokyol C et al. Bacterial vaginosis: comparison of pap smear and microbiological test results. *Modern Pathology*. 2004; 17:857-60
2. Marchiano, D. Infections in pregnancy: bacterial vaginosis. Healthline, 2012. <http://healthline.com/health/pregnancy/infections-asymptomatic-bacteruria>
3. Clinical Effectiveness group, British Association for Sexual Health and HIV (BASHH). National guideline for the management of bacterial vaginosis. London (UK): British Association for Sexual Health and HIV (BASHH); 2006 www.bashh.org/documents/4413.pdf
4. Brocklehurst P, Gordon A, Heatley E, Milan SJ. Antibiotics for treating bacterial vaginosis in pregnancy. *Cochrane database of systematic reviews* 2013, Issue1. Art No. CD000262
5. Mark H. Yudin, MD and Deborah M. Money, MD. Screening and management of bacterial vaginosis in pregnancy. *SOGC Clinical Practice Guideline*. No 211. August 2008
6. 2010 Centers for Disease Control and Prevention (CDC) Sexually Transmitted Disease (STD) Treatment guidelines www.cdc.gov/std/
7. Michael, Claire MD. The papanicolaou smear and the obstetric patient: a simple test with great benefits. *Diagnostic cytopathology*. 1999; Vol 21-1
8. Lamont, RF et al. A comparison of the use of papanicolaou-stained cervical cytological smears with gram-stained vaginal smears for the diagnosis of bacterial vaginosis in early pregnancy. *Int J STD AIDS*. 1999 Feb;10(2):93-7
9. Lipana, Ma Katherine, MD. *Accuracy of the Papanicolaou smear in the Diagnosis of Bacterial Vaginosis*. 2006
10. Raina, A et al. Pap smears in the diagnosis of Bacterial Vaginosis. *Indian Medical Gazette*. 2013
11. Nelson, Deborah and Macones, George. Bacterial vaginosis in Pregnancy: Current Findings and Future Directions. *Oxford Journal of Medicine*. 2002 Volume 24 Issue 2. pp 102-108
12. Lata, I et al. Estimation of the incidence of bacterial vaginosis and other vaginal infections and its consequences on maternal/fetal outcome in pregnant women attending an antenatal clinic in a tertiary care hospital in north india. *Indian J Community Med*. Apr 2010; 35(2): 285-289.
13. Walker, J et al. The prevalence and incidence of bacterial vaginosis in a cohort of young Australian women. *Sex Transm Infect* 2011; 87: A110-A111
14. Mengistie, Z et al. Comparison of the clinical and gram stain diagnosis methods of bacterial vaginosis among pregnant women in Ethiopia. *J Clin Diagn Res*. 2013 Dec; 7(12):2710-3
15. Mass SB, Brennan JP, Silverman N, Van Hoeven KH. Association between a shift in vaginal flora on Papanicolaou smear and acute chorioamnionitis and preterm delivery. *Diagn Cytopathol*. 1999; 21:7-9.
16. Leukorrhea during pregnancy at pregnancy weekly, 2012. www.pregnancy-blog.parentingweekly.com/2012/06/leucorrhea-during-pregnancy.html
17. Og A, Oe O, To A. Sensitivity of a papanicolaou smear in the diagnosis of candida albicans infection of the cervix. *North Am J Med Sci* 2010; 2: 97-99.
18. Berek, JS. Simplification of the new Bethesda 2001 classification system. *Am J Obstet Gynecol* 2003; 188: S2-5.
19. Akobeng, Anthony. Understanding diagnostic tests1: sensitivity, specificity and predictive values. *Acta Paediatrica*. 2006
20. Attia, John. Moving beyond sensitivity and specificity: using likelihood ratios to help interpret diagnostic tests. *Australian Prescriber* Vol 26 No 5 2003
21. Karani A et al. The Pap smear for detection of bacterial vaginosis. *International Journal of Gynecology and Obstetrics* 2007; 98:20-30
22. Sodhani P et al. Prevalence of bacterial vaginosis in a community setting and role of the pap smear in its detection. 2005 Nov-Dec; 49(6):634-8
23. Vardar E et al. Comparison of Gram stain and Pap smear procedures in the diagnosis of bacterial vaginosis. *Infect Dis Obstet Gynecol*. 2002; 10(4): 203-207
24. Heather Yun MD. *Bacterial Infections in Pregnancy*. Medscape. February 2011
25. Tam, M.T., MYungbluth and T. Myles. Gram stain method shows better sensitivity than clinical criteria for detection of bacterial vaginosis in surveillance of pregnant, low income women in a clinical setting. *Infectious Diseases in Obstetrics and Gynecology*. 1998; 6:204-208
26. Prey M. Routine Pap smears for the diagnosis of bacterial vaginosis. *Diagn Cytopathol* 1999; 21:1013.