Prevalence and Risk Factors Associated with Bacterial Vaginosis Among Women Seen at the Out-Patient Department of a Tertiary Medical Center in 2017 to 2019: A Hospital-Based Experience

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ABSTRACT

Background: Bacterial vaginosis (BV) is the leading cause of vaginal infections affecting approximately three million women yearly worldwide. The prevalence was noted to increase through the years. Varying literatures showed conflicting evidence as to how the change in the abnormal vaginal flora comes about.
Objective: To determine the prevalence and risk factors associated with bacterial vaginosis among women seen at the outpatient department of a tertiary medical center.
Methods: This is a cross-sectional study which involved review of patients' charts of women, 18 to 44 years old, who were seen at the outpatient department of a tertiary medical center from 2017 to 2019 presenting with homogenous, white to grayish vaginal discharge. Diagnosis of BV was based on Nugent's score on gram stain with a score of 4 to 6 with clue cells or a score of 7 to 10. Purposive sampling was done. Data gathered included age, gravidity, parity, pregnancy status, educational status, coitarche, number of sexual partners, family planning method, comorbidities, smoking and alcohol consumption.
Results: Prevalence of bacterial vaginosis from 2017 to 2019 in this institution accounts to 70.57% (271/384). The risk factors found to be significantly associated with bacterial vaginosis were age (p 0.003), parity (p 0.001), pregnancy status (p 0.043), number of sexual partners (p 0.002), use of family planning method (p < 0.001), presence of comorbidities (p <0.001), smoking (p 0.003), and alcohol consumption (0.014).
Conclusion: The prevalence of bacterial vaginosis in this institution is higher than those previously reported. Women at higher risk for bacterial vaginosis are multiparous, non-pregnant, with 2 or more sexual partners, comorbidities, using barrier method and IUD contraceptives, smoker as well as alcohol-beverage drinker. The risk of BV is lower among woman aged 18 to 30 years old.

Key words: bacterial vaginosis, prevalence, risk factors, sociodemographic factors and sexual behaviors

Bacterial vaginosis (BV) is a common condition characterized by an abnormal alteration in the vaginal flora. Lactobacilli represent 95% of bacteria in the vagina maintaining an acidic environment and preventing growth of other microbial organisms. In BV, Lactobacilli is reduced and the concentration of other bacteria increases by 100 to 10,000 fold. This leads to its usual manifestation of a homogenous foul-smelling vaginal discharge presented by 29% of women.[1] Although, 50% of cases are asymptomatic, it is the leading cause of vaginal infections affecting approximately three million women yearly. In the Philippines, cases of BV in pregnant women is approximately 12 to 22%. On the other hand, its prevalence among non-pregnant women is about 28%.[2] In the recent years, some literatures reported the prevalence to be increasing 29.2 % in 2001 to 2004 in a study by Koumans, et al.[3] to 39.5% in another study by Bitew, et al. in 2019 to 2020.

The first line therapy in treating BV includes seven days of oral metronidazole. Although proven effective, the limiting factor to its use is the occurrence of adverse effects more commonly gastrointestinal in nature such as nausea, vomiting and metallic taste and the emergence of resistance leading to recurrence.

Alternative regimens according to Centers for Disease Control and Prevention (CDC) on their 2015 Sexually Transmitted Diseases Treatment Guidelines are tinidazole, oral or intravaginal clindamycin and extended-release oral tablets of metronidazole.

Despite its public health importance and high number of cases, pathogenesis of BV remains unclear. Disagreement exists as to how the change in the abnormal vaginal flora comes about.[4] Is it due to the introduction of multiple pathogens in the vagina or do other risk factors lead to overgrowth of BV-associated microorganisms? According to literatures, there are certain factors that affect vaginal ecosystem. The factors that were identified include ethnicity, douching, multiple or new sexual partner and smoking.[5] These factors may cause an overgrowth of the BV- associated organisms. Most of the literatures regarding risk factors were conducted in Western countries, and were not found to be consistent.

Hence, this study would like to investigate the prevalence of BV and its associated risk factors in women in a hospital-based setting. The rationale in determining the related factors is to find preventive strategies for bacterial vaginosis that would thereby translate to improve women's health. Determining the triggers or risk factors for the development of BV is crucial in formulating strategies for prevention as well as improving treatment schemes among women with BV.

Review of Related Literature

Bacterial vaginosis (BV) is one of the most prevalent infections in women of reproductive age. Etiology behind this condition is known to be related to an altered normal vaginal flora with reduction in the Lactobacillus population and an overgrowth of anaerobic bacteria including Gardnerella vaginalis, Bacteroides sp., Prevotella sp., Mobiluncus sp., Fusobacterium nucleatum, Peptostreptococcus sp., Ureaplasma urealyticum and Mycoplasma hominis.[3] Bacterial vaginosis is usually present in 30 to 50% of sexually active women complaining of a homogenous fishy odor vaginal discharge. This condition is easily treatable but if left undiagnosed and untreated may cause significant obstetrics and gynecologic sequelae. The adverse pregnancy outcomes that has been associated with BV include miscarriage, premature rupture of fetal membranes (PROM), preterm labor and delivery, chorioamnionitis, endometritis and neonatal complications. In non-pregnant women, it has been reported to cause upper genital infections such as cervicitis, pelvic inflammatory disease (PID), urinary tract infections (UTI), cervical intraepithelial neoplasia (CIN) and a higher risk for human immunodeficiency virus infection. These adverse outcomes for both pregnant and non-pregnant women occur as a result of ascending genital infection from the vagina through the cervix to the uterine cavity.[1]

Diagnosis is achieved using Amsel's criteria or Gram stain-based Nugent score. Clinical diagnosis of BV is made when at least 3 of the following 4 components of the Amsel's criteria are present which include thin homogenous vaginal discharge, vaginal pH of greater than or equal to 4.5, positive amine or whiff test using 10% KOH and presence of clue cells more than 20% on microscopy. The Amsel's criteria do not include assessment of vaginal Lactobacilli and was developed for evaluation of symptomatic women. To confirm the diagnosis of BV a microscopic diagnosis according to the Nugent score must be done. This is dependent on the gram stain findings of the vaginal discharge. A score of 0 to 3 reflects the presence of normal flora (dominated by 1 to two species of Lactobacillus), 4 to 6 as intermediate flora and 7 to 10 representing BV.[6] This scoring method is utilized more on researches because of improved quality assurance and reproducibility of results.

Antibiotic therapy is the recommended first line treatment for BV. This aims to decrease the anaerobes without affecting the Lactobacilli. Treatment options include either topical or oral formulation of Metronidazole or Clindamycin. The gold-standard is the seven-day course of oral Metronidazole 500 mg twice a day. The alternative treatment regimens include oral clindamycin 300 mg twice a day for seven days, intravaginal 2 % clindamycin cream once daily for seven days, or an intravaginal metronidazole gel once daily for five days.[7] However, clindamycin cream and intravaginal metronidazole gel are not available in the Philippines.

The natural history of BV is still not certain, as well as the risk factors for its acquisition. There are numerous potential risk factors identified. One of this is race wherein blacks have higher rates than whites. Racial difference in vaginal microbiology may be a possible explanation for this discrepancy or may be due to culturally-mediated differences in vaginal cleansing behaviors such as douching. Smoking, alcohol consumption, use of certain family planning methods, lower socio-economic status and lower educational levels have also been associated with BV.

Data regarding the association between these factors even with sexual activity and BV are contradictory and still lack clear evidences.[4]

To be able to gather the evidence on the prevalence of BV and its associated risk factors, a computerized literature search was done. The following search terms were used: bacterial vaginosis, prevalence, risk factors, sociodemographic factors and sexual behaviors. The union and intersection of the free text and MeSH terms of these terms yielded 316 articles. Among these, only 17 studies were relevant and all of which are individual studies. Hand search was also done obtaining 3 studies. With these, 15 studies are related to prevalence and risk factors of BV in non-pregnant women while 5 are associated with pregnancy. In addition, hand search through the Philippine Obstetrical and Gynecological Society database was made which yielded no similar article.

In the meta-analysis done by Kenyon, et al.[5] in 2013, the global epidemiology of BV was described. Their search was conducted using PubMed/Medline and Web of Science databases. Search terms included "bacterial vaginosis", "epidemiology", "incidence" and "prevalence". This resulted in the retrieval of 1692 articles. There were 86 articles involving 9288 participants that met the inclusion criteria. BV prevalence was found to vary considerably between ethnic groups in North America, South America, Europe, Middle East and Asia. Comparison of BV prevalence by geographic area are hampered by a number of factors including how samples were selected and differences in the type of population surveyed. Despite these, certain trends are apparent. In Southern and Eastern Africa, prevalence of BV was highest at 68.3 % with a slight predilection on the older age group. BV prevalence tended to be intermediate in the region of Latin America and Caribbean. Two populations, in Jamaica and a rural area in Peru, had rates of 49 and 41%, respectively. On the other hand, there was a paucity of data from the regions of Middle East and North Africa. In North America, East, South and Southeast Asia, prevalence were lowest with mean rate at 30%.[5]

Another systematic review and meta-analysis by Peebles, et al.[8] in 2019 was conducted to determine the global BV prevalence among reproductive-aged women in the general population. PubMed and Embase were used as search engines with 2361 records identified. There were 122 relevant studies available for extraction on prevalence involving 12095 participants. They also used random effects models to estimate BV prevalence by global regions. General population prevalence of BV is high globally, ranging from 23% to 29% across regions. Prevalence in percentage were as follows: Europe and Central Asia, 23%; East Asia and Pacific, 24%; Latin America and Caribbean, 24%; Middle East and North Africa, 25%; sub-Saharan Africa, 25%; North America, 27%; and South Asia, 29%. Within North America, black and Hispanic women have significantly higher at 33% and 31%, respectively, in comparison with other racial groups. Further researches to determine the etiology of BV and corresponding prevention and sustainable treatment strategies are urgently needed to reduce the burden of BV among women.[8]

A number of social and sexual risk factors for BV had been recognized. However, there is still no definite consensus regarding their association. Holzman, et al. in 2001 tested the hypothesis that vaginal douching is linked with BV in both symptomatic and asymptomatic women and also identified other demographic, reproductive and lifestyle factors associated with BV. A cross-sectional study was done involving 3 clinic sites with 496 non-pregnant women aged 18 to 40 years old who completed self-administered questionnaires. Their vaginal smears were assessed and cross-validated for BV. Of which, 107 participants were diagnosed. The prevalence of BV across clinics ranged 15 to 30%. Results showed that BV remained significant for African American women with 13 or fewer years of education (Odds Ratio [OR] = 5.5, 95 % Confidence Interval [CI] = 2.1, 14.5), hormone use within the past 6 months (OR = 0.5, 95 % CI = 0.2,0.8) and vaginal douching within the past 2 months (OR = 2.9, 95 % CI = 1.5, 5.6). They've concluded that two lifestyle factors were strongly associated with BV: systemic contraceptives appear protective whereas douching increases its risk. However, the temporal relationship between douching and BV needs further clarification.[9]

Smart, et al. in 2004 aimed to clarify potential sexual and non-sexual risk factors for BV. Women from Sydney Health Center with BV between March 1991 to July 1999 were included. Controls were randomly selected women without BV. Information regarding demographics, clinical findings, sexual and non-sexual risk behaviors were extracted from the clinical database. A logistic regression model was use to establish which associations with BV persisted. Results showed that of 15,567 women who were seen during the study period, 890 were

diagnosed with BV and 890 controls were studied. Factors that were independently associated with BV were more than 3 male sexual partners in the past 12 months (Odds Ratio (OR) = 1.60, 95% Confidence Interval (CI): 1.19 to 2.04), at least one female sexual partner in the past 12 months (OR = 2.1, p = 0.003), a past pregnancy (OR = 1.5, p,0.0006), and smoking. In contrast, women with BV were significantly less likely to have used hormonal contraception (OR = 0.60, 95% CI: 0.51 to 0.81) or to have used condoms consistently (OR = 0.5, 95% CI: 0.31 to 0.71) than controls. The limitation of this study is that although a number of factors were found to be associated with BV, the small odd ratios suggest that the individual predictive value may be low.[10]

In 2007, Jones, et al. determined the prevalence of BV from a socioeconomically deprived population. They also determined the association of BV with risk factors for sexually transmitted diseases (STDs). There were 965 women who answered an epidemiologic survey determining sexual risk behaviors. Biologic samples from the participants were obtained to test for BV and STDs. Results showed that the prevalence of BV was high at 27 % and was significantly associated with having a bacterial STD or trichomoniasis. On the other hand, many sexual behaviors including being sexually experienced, mean number of lifetime sexual partners and mean number of unprotected sexual intercourse showed no significant association with BV. The limitation of this study was that the survey used assessed general sexual conduct and did not examine specific risk factors for BV such as douching or birth-control methods.[11]

Moreover, Om, et al. (2015) aimed also to determine the association of risk factors for BV patients. A prospective study was done including 300 non-pregnant women. A total of 156 were tested positive for BV confirmed by Amsel's Criteria and Nugent's Score. All participants were interviewed using a predetermined questionnaire. Information on age, age of marriage, use of intrauterine device, oral contraception, vaginal douching, use of condom, number of sexual partners, STD in partners, occupation and region of origin were recorded. Results showed that frequency of intrauterine contraceptive device differed significantly between BV and healthy individuals (29.4% versus 17.2%, p=0.017; OR=1.70). Furthermore, participants with a sexually transmitted disease in partners conferred significantly higher risk for BV (p=0.001, OR=8.28). However, use of contraception, vaginal douching, and use of condom did not modulate the risk of BV. Individuals with the use of intrauterine contraceptive device at 24% and presence of sexual transmitted disease in partners at 12% conferred a higher risk for bacterial vaginosis. Further studies with larger sample size is recommended to screen the risk factors associated with BV.[12]

Similarly, Ranjit, et al. in 2018 assessed the association of the risk factors with BV. A descriptive cross-sectional study was conducted among 160 non-pregnant women tested for BV using Nugent's Scoring. The overall prevalence was 24.4%. Questionnaires were also used to collect sociodemographic, behavioral, medical, reproductive and sexual history. Douching was statistically related to BV (p = 0.015). Contraceptives users on anatomical sites were found to be more prone to BV due to imbalance in vaginal flora. The risk of BV was higher among those who used condom daily at 42.9% and those with intrauterine device at 30%. For contraceptive users on non-anatomical sites like those of oral contraceptive pills, prevalence was least at 9.1%. The prevalence of BV was found higher in daily smokers of 30% while 0% was reported among occasional smokers. Daily alcohol users had the highest prevalence of BV of 38.5% while the least prevalence of 24% was recorded among those who never drank alcohol.[13]

Among the studies mentioned, the common denominator related to BV is douching. Furthermore, there were also few studies and guidelines paying attention on the topic of intimate female hygiene. A cohort study by Klebanoff, et al. (2010) evaluated the associations between BV and hygienic behavior. There were 3620 women with BV recruited for the study who completed 13,587 study visits. At each visit, a face to face interview was conducted. The questions specifically asked were family planning used, frequency of showering and or bathing, frequency of use of feminine hygienic products such as vaginal wash, wipes, spray or suppositories, and frequency of douching. The presence of menstruation, use of specific menstrual products like pads and tampons and sanitary napkin or panty liners when not menstruating as well as the type of underwear commonly used were all ascertained as well. Results showed that personal hygienic behaviors practiced that were not related to douching were not strongly associated with recurrent BV. On the other hand, douching remained associated with BV. With regards to the method of family planning used, hormonal

contraception has a BV prevalence of 34% while those who underwent tubal ligation has a greater prevalence at 49.6%. One limitation of the study was that it did not collect data on specific composition of the oral contraceptives such as those oral contraceptives containing progestin only. Thus, the role of specific hormones in BV cannot be explored. Other limitations were the following: type of underwear and use of pads and pantyliner were assessed only at baseline; data on brand of tampons or pads were not collected; and question on vaginal suppositories were non-specific. Therefore, the link between BV and these behaviors may have been misclassified. In contrast, the strengths of the study include the questionnaire used with an extensive array of behaviors being evaluated and the large sample size of the study.[14]

Additionally, during pregnancy, BV is known to be present in 20% of women and majority of cases are asymptomatic. The nature of this condition usually resolved spontaneously without any treatment. However, diagnosis of BV in early pregnancy is more likely to persist and if not treated may be associated with adverse outcomes. Meta-analysis regarding adverse effects of BV in pregnancy includes 32 studies involving 30,000 pregnant women. Analysis demonstrated that BV doubled the incidence of preterm delivery in patients without symptoms. It also increased risk of late miscarriages and maternal infections.[15] In another study, 59.6% out of 200 pregnant women with BV developed adverse maternal and/or fetal outcome. Late miscarriage was increased by 7.5-fold while preterm labor and delivery by 3.22-fold.[16] Bacterial vaginosis in the second trimester of pregnancy was found to have higher risk of preterm delivery and PROM accounting to 83 % of cases.[17]

Achdiat, et al. in 2019 investigated on the prevalence of BV among pregnant women. A cross-sectional study was done involving 60 pregnant women aged 17 to 40 years old. Out of all the participants, seven or 11.67% had BV according to the Amsel's criteria. However, asymptomatic BV was diagnosed in all participants. In this study, out of the 9 participants in the first trimester, 2 experienced BV. Out of the 37 in the second trimester, also 2 developed BV. On the other hand, 3 out of the 14 participants in the third trimester had BV. Based on these, BV is more likely to developed on the first and last trimesters of pregnancy. To avoid maternal and fetal complications associated with this condition, it should not be waited for the symptoms to reveal. Thus, they recommend screening of BV as a routine work-up.[18]

On the same year, Kamga, et al. determined the prevalence and risk factors of BV among pregnant women. A structured questionnaire was administered to 309 pregnant women to gather data on demographic, obstetric and gynecological characteristics and hygiene behavior. Vaginal smear was also collected for gram staining. Results showed that prevalence of BV was 26.2% based on Nugent's score. BV was higher about 29.5% in participants from the rural area (Chi-square test [X^2] = 8.609, p = 0.014), those who did not use antibiotics prior to the study in 31.9% (X^2= 12.893, p = 0.002) and women with no history of a genital tract infection (X^2 = 18.154, p = 0.001). There was a significant difference in prevalence with respect to gestation age (X^2 = 13.959, p = 0.007) with the highest occurring in women in the second trimester of 31.7%. Women who practiced douching (X^2 = 23.935, p = 0.000) and those who did not wash pants with disinfectant (X^2 = 7.253, p = 0.027) had a high prevalence.[19]

Based on evidence available, vaginal douching and use of intrauterine contraceptive device were constantly associated with BV. However, previous studies showed limitation in the sample size. Moreover, there are limited literatures regarding BV and its associated risk factors during pregnancy. It is therefore worthwhile to explore these aspects to improve the prevention of BV among women of different ages and pregnancy status.

Significance of the Study

Little is known about the natural history of BV. Moreover, its etiology and pathogenesis remain poorly understood. Few studies have determined prevalence of BV and its associated risk factors among Asian women. Previous studies done in other countries have identified a number of risk factors and behaviors associated with BV in both pregnant and non-pregnant population including number of sexual partners, lower age of first intercourse, being unemployed, being unmarried, cigarette smoking and use of certain forms of contraception. However, many of these studies included small number of patients or had poorly defined patient population. This study aimed to investigate the potential sexual and non-sexual risk factors for BV in an urban outpatient department of a tertiary hospital in the Philippines.

Preventive strategies usually target the risk factors or behaviors for the disease. Hence, knowledge on the prevalence and possible risk factors associated with

BV can be used as a basis in formulating strategies to prevent occurrence of BV and consequently avoid its adverse outcomes for both pregnant and non-pregnant women. Overall, this can be contributory in lowering the incidence and associated healthcare cost of BV. Thereby, the results of this study may contribute to improve the health status of women.

The objective of the study was to determine the prevalence and risk factors associated with bacterial vaginosis among women seen at the outpatient department of a tertiary medical center in the past three years from 2017 to 2019.

MATERIALS AND METHODS

Study Design

A retrospective cross-sectional study was done and determined the prevalence of BV and identified the risk factors associated to its occurrence.

Setting and Population

The study reviewed charts of women 18 to 44 years old who consulted at the outpatient department of a tertiary medical center due to vaginal discharge from the year January 2017 to December 2019.

Chart records of women with the following characteristics were included in the study (Inclusion Criteria):

1. Presented with homogenous, white to grayish vaginal discharge
2. Diagnosed with BV based on Nugent's score: Gram Stain Result (Nugent's Score) of 4 to 6 with Clue Cells or Score of 7 to 10
3. If pregnant, those with initial check-up within 8 to 40 weeks

Chart records of women with the following characteristics were excluded in the study (Exclusion criteria):

1. Other known vaginitis, such as Candidiasis and Trichomoniasis
2. Antibiotic therapy within 2 weeks
3. Bleeding from the genital tract of unknown etiology

Methodology

In this three year (January 2017 to December 2019) review of the charts of patients, the eligible records underwent data extraction by the primary investigator. The data obtained was gathered using a standard data collection tool (See Appendix I). The data extracted include the age, gravidity, parity, pregnancy status, age of gestation (if pregnant), educational status, coitarche, number of sexual partners, family planning method used, comorbidities present, smoking and alcohol consumption. Purposive sampling was done.

Sample Size

The sample size calculation was based on the formula of single proportion.

The first sample size calculation was based on the reported prevalence of BV in this institution from 2017 to 2019. The proportion P is 22 %. Using 1.96 (z) as the standard error associated with 95% confidence interval and a desired precision of 0.05, the required sample size for this study is 264.

The next sample size calculation was based on age at increased risk for BV are those aged 18 to 30 years old. The proportion p is 14%.[14] Using 1.96 (z) as the standard error associated with 95% confidence interval and a desired precision of 0.05, the required sample size for this study is 186.

The third sample size calculation was based on gravidity at increased risk for BV are the multigravida. The proportion p is 30.9%.[20] Using 1.96 (z) as the standard error associated with 95% confidence interval and a desired precision of 0.05, the required sample size for this study is 329.

The fourth sample size calculation was based on parity at increased risk for BV are the multipara. The proportion p is 42%.[10] Using 1.96 (z) as the standard error associated with 95% confidence interval and a desired precision of 0.05, the required sample size for this study is 375.

The fifth sample size calculation was based on age of gestation at increased risk for BV which is at the second trimester. The proportion p is 31.7%.[20] Using 1.96 (z) as the standard error associated with 95% confidence interval and a desired precision of 0.05, the required sample size for this study is 333.

The sixth sample size calculation was based on educational attainment at increased risk for BV which are those with no formal educational attainment. The proportion p is 55%.[14] Using 1.96 (z) as the standard error associated with 95% confidence interval and a desired precision of 0.05, the required sample size for this study is 380.

The seventh sample size calculation was based on age of coitarche at increased risk for BV which is from 18 to 20 years old. The proportion p is 50%.[19] Using 1.96 (z) as the standard error associated with 95% confidence interval and a desired precision of 0.05, the required sample size for this study is 384.

The eighth sample size calculation was based on the number of sexual partners at increased risk for BV which is having more than two partners. The proportion p is 32 %.[10] Using 1.96 (z) as the standard error associated with 95% confidence interval and a desired precision of 0.05, the required sample size for this study is 335.

The ninth sample size calculation was based on the family planning method used at increased risk for BV which is condom. The proportion p is 42.9%.[14] Using 1.96 (z) as the standard error associated with 95% confidence interval and a desired precision of 0.05, the required sample size for this study is 377.

The tenth sample size calculation was based on comorbidity at increased risk for BV which is presence of diabetes mellitus. The proportion p is 25.20%.[21] Using 1.96 (z) as the standard error associated with 95% confidence interval and a desired precision of 0.05, the required sample size for this study is 290.

The eleventh sample size calculation was based on smoking habit and at increased risk for BV are the smokers. The proportion p is 30%.[14] Using 1.96 (z) as the standard error associated with 95% confidence interval and a desired precision of 0.05, the required sample size for this study is 323.

The last sample size calculation was based on alcohol consumption and at increased risk for BV are those who are non-alcoholics. The proportion p is 42.8%.[7] Using 1.96 (z) as the standard error associated with 95% confidence interval and a desired precision of 0.05, the required sample size for this study is 377.

Among the sample size calculated, the authors chose the largest which was based on the age of coitarche. Hence, a total of 384 charts of women with BV from 2017 to 2019 were included in this study.

Data Analysis and Management

Data were encoded and entered in Microsoft Excel. Analysis of data was done using Stata version 9.0. Univariate analysis such as mean and range was used to describe continuous variables such as age, parity, age of gestation (AOG), age of coitarche and number of sexual partners. Frequency Distribution was utilized to describe categorical variables such as age group, gravidity and parity group, AOG group, age of coitarche group, number of sexual partner group, educational status, family planning method, presence or absence of comorbidities, history of smoking and/or alcohol consumption as well as the trimester category among pregnant cases.

The T-test was used in the analysis of the association of the continuous variables with BV. On the other hand, the association of categorical variables with BV was analyzed using Chi-square or Fisher's exact test. Logistic regression was done to further determine the association of the factors with BV. The study utilized 0.05 level of significance.

Ethical Considerations

This study was implemented after the approval of the technical and ethics review board of the institution. Permission to retrieve and review the records was obtained from the medical records. Hence, the informed consent was waived in this study due to its retrospective nature. No interaction and no direct risks were acquired by the patient.

Information of the participants as well as the outcome of the study were kept confidential. Anonymity was maintained by assigning number codes per participant data collection tool. There was no identifying mark nor clue regarding the patient in any part of the data collection tool and the results section of the paper. The data gathered were stored in a computer file that is password-protected, while the data collection tool and master list were kept in a passcode protected cabinet. Only the primary investigator had access to these files. The files will be stored in the password protected computer for 5 years and will be deleted while the master list will be shredded thereafter. There was no conflict of interest involved in this study.

RESULTS

There were 384 cases of women aged 18 to 44 years old with vaginal discharge seen at the outpatient department from January 2017 to March 2020 with chart records eligible for review. Out of the 384 cases, there were 271 cases (70.57 %) diagnosed with BV using Nugent's score on gram stain. Among the cases reviewed, the risk factors were compared between those who had and did not have BV.

When grouped according to age, there were 50.18% (136/271) among charts of women aged 18 to 30 years old and 49.82 % (135/271) aged 31 to 44 years old with BV. For this variable, the difference between them was statistically significant (p value <0.001). There was a significantly lower percentage of charts with BV in the 18 to 30 years old age group as compared to those without (72.57%). (Table 1)

For the gravidity, among those with BV 13.28% (36/271) were nulligravid, 29.15% (79/271) were primigravid and 57.56 % (156/271) were multigravid. There was no statistically significant difference with this variable (p value 0.057) between those with and without BV. When grouped according to parity, for those with BV 32.84% (89/271) were nullipara, 26.19% (71/271) primipara and 40.96% (111/271) multipara. It is only the risk factor on parity that showed statistically significant difference (p value of 0.001). It was the charts of multiparous women having the highest proportion with BV while near half (46.9%) of those without BV were charts of nulliparous women.

Among non-pregnant women, about 54.98% (149/271) were diagnosed with BV as compared to 45.18% of those who were pregnant. For pregnant women with BV, about 12.92% (35/271) were diagnosed in the first trimester, 18.45% (50/271) in the second trimester and 13.65% (37/271) in the third trimester. There was a statistically significant difference in the proportion of women who developed BV with the pregnancy status variable (p value of 0.043). Overall, there was a significantly higher percentage of BV among non-pregnant case (54.18%) as compared to those pregnant cases (45.02%).

In terms of educational status, around 5.53% (15/271) of cases with BV had no formal education or were elementary undergraduates. The cases of BV among women who were elementary graduates were 28.04% (76/271), for those who were high school graduates about 48.71% (132/271) while there were 17.71% (48/271) among the college graduates. With regards to educational status variable, the difference in the proportion of women with BV were not statistically significant (p value 0.170).

Regarding the variable on age of coitarche. There were comparable number of cases (p value 0.678) with BV among those with coitarche at less than 20 years old at 52.77% (143/241) and between 20 to 44 years old at 47.23% (128/271). For the number of sexual partners, among those cases with BV, there were 31.37% (85/271) having one partner while 68.63% (186/271) have 2 or more partners. The difference in the proportion of cases of women with BV based on the number of sexual partners as a risk factor was statistically significant (p value of < 0.001). There was a significantly higher proportion of cases with BV among the charts of women with two or more sexual partners while a significant higher proportion of cases with one sexual partner does not have BV.

The use of family planning method as a risk factor was categorized according to the type of method. Among those with BV, there were 50.55% (137/271) cases who have no family planning method utilized, while 49.95% (134/271) were on contraceptives. Among those on contraception with BV, 4.80% (13/271) used calendar method, 20.66% (56/271) were condom users, 9.96% (27/271) were using combined oral contraceptives, 9.59% (26/271) were on progestin-only contraceptives, 2.95% (8/271) were IUD users and 1.48% (4/271) underwent BTL. The highest proportion of cases with BV among those using family planning method were those on condom use. However, among the 8 cases with IUD and the 4 cases who underwent BTL, all of them had BV. The difference between the proportion of cases with BV according to the type of family planning method was statistically significant (p value 0.001).

For the comorbidities, in patients with BV, there were 50.92% (138/271) of cases who had no comorbidities, 9.59% (26/271) were hypertensive, 10.70% (29/271) were diabetic, 3.69% (10/271) with thyroid disease, 9.96% (27/271) with pulmonary disease, 1.85% (5/271) with kidney disease, 1.48% (4/271) with malignancy, 11.07% (30/271) with combination of two or more conditions and 0.74% (2/271) with combination of three or more conditions. There was a statistically significant difference in the proportion of cases with or without BV across the comorbidities (p value < 0.001). A significantly higher percentage of cases with BV were noted among the charts reviewed with single or combination of co-morbidities as compared to those without BV. Among those without BV, the highest proportion were significantly noted among those without co-morbidities.

With regards to smoking as a factor, among the cases with BV, about 11.81% (32/271) were smokers and 88.19% (239/271) were non-smokers. Among the charts of women who smoke, around 32 out of 33 had BV. Hence, when compared to non-smokers (0.88%), there was statistically significant higher proportion of cases with BV among those who smoke (p value 0.001).

Lastly, for alcohol consumption, among cases with BV, there were 16.61% (45/271) who were alcoholic beverage drinkers. Out of 53 cases of women who were alcoholic-drinkers, 45 of them developed BV. This showed a statistically significant higher proportion of BV among alcoholic beverage drinkers as compared to non-alcoholic drinkers (7.08%) with p value of 0.014. (Table 1)

Table 1. Comparison of the risk factors associated with bacterial vaginosis.

	(+) BV N=271 n (%)	(-) BV N=113 n (%)	p value
Age			<0.001
18-30	136 (50.18)	82 (72.57)	
31-44	135 (49.82)	31 (27.43)	
Gravidity			0.057
Nulligravid	36 (13.28)	19 (16.81)	
Primigravid	79 (29.15)	44 (38.94)	
Multigravid	156 (57.56)	50 (44.25)	
Parity			0.001
Nullipara	89 (32.84)	53 (46.90)	
Primipara	71 (26.19)	37 (32.74)	
Multipara	111 (40.96)	23 (20.35)	
Pregnancy Status			0.043
Non-Pregnant	149 (54.98)	54 (47.79)	
Pregnant	122 (45.02)	59 (52.21)	
1st Trimester	35 (12.92)	21 (18.58)	
2nd Trimester	50 (18.45)	22 (19.47)	
3rd Trimester	37 (13.65)	16 (14.16)	
Educational Status			0.170
No Formal Education or Elementary Undergraduate	15 (5.53)	1 (0.88)	
Elementary School Graduate	76 (28.04)	34 (30.09)	
Highschool Graduate	132 (48.71)	61 (53.98)	
College Graduate	48 (17.71)	17 (15.04)	
Coitarche			0.678
<20 years old	143 (52.77)	57 (50.44)	
20 to 44 years old	128 (47.23)	56 (49.55)	
Number of Sexual Partners			<0.001
1 Partner	85 (31.37)	68 (60.18)	
2 or More Partners	186 (68.63)	45 (39.82)	
Family Planning Method			0.001
None	137 (50.55)	79 (69.91)	
Contraceptive User	134 (49.45)	31 (30.09)	
Calendar Method	13 (4.80)	11 (9.73)	
Condom	56 (20.66)	12 (10.62)	
Combined Oral Contraceptives	27 (9.96)	6 (5.31)	
Progestin-only Contraceptives (Oral/Implant/Injectable)	26 (9.59)	5 (4.42)	
Intrauterine Device	8 (2.95)	0 (0)	
Bilateral Tubal Ligation	4 (1.48)	0 (0)	
Comorbidities			<0.001
None	138 (50.92)	98 (86.73)	
Hypertension	26 (9.59)	4 (3.54)	
Diabetes Mellitus	29 (10.70)	2 (1.77)	
Thyroid Disease	10 (3.69)	1 (0.88)	
Pulmonary Disease	27 (9.96)	6 (5.31)	
Kidney Disease	5 (1.85)	0 (0)	
Malignancy	4 (1.48)	0 (0)	
Combination of Two Conditions	30 (11.07)	2 (1.77)	
Combination of 3 or More Conditions	2 (0.74)	0 (0)	
Smoking			0.001
Smoker	32 (11.81)	1 (0.88)	
Non-smoker	239 (88.19)	112 (99.11)	
Alcohol			0.014
Alcoholic Drinker	45 (16.61)	8 (7.08)	
Non-alcoholic Drinker	226 (83.39)	105 (92.92)	

When adjusted to all other risk factors, the same factors were found to be most associated with BV. These include age group (OR = 0.347, 95% CI 0.172 to 0699), parity (OR = 1.08, 95% CI 0.535 to 2.19), pregnancy status (OR = 0.715, 95% CI 0.517 to 0.989, number of sexual partners (OR = 0.427, 95% CI 0.248 to 0.735), use of family planning method (OR = 0.548, 95% CI 0.432 to 0.695), comorbidities (OR = 0.628, 95% CI 0.518-0.762), smoking (OR = 4.150, 95% CI 1.634 to 10.54) and alcohol consumption (OR = 2.346, 95 % CI 1.071 to 5.134). (Table 2)

Table 2. Multivariate analysis of risk variables for bacterial vaginosis

Factor	Odds Ratio (95 % CI)	p-value
Age group	0.347 (0.172 to 0.699)	0.003
Gravidity group	0.966 (0.451 to 2.068)	0.928
Parity group	1.084 (0.535 to 2.196)	0.001
Pregnancy status group	0.715 (0.517 to 0.989)	0.043
Educational status group	1.049 (0.736 to 1.495)	0.793
Coitarche group	1.545 (0.836 to 2.856)	0.165
Number of Sexual Partner group	0.427 (0.248 to 0.736)	0.002
Family Planning Method group	0.548 (0.431 to 0.695)	< 0.001
Comorbidities group	0.628 (0.518 to 0.761)	< 0.001
Smoking group	4.151 (1.634 to 10.538)	0.003
Alcohol group	2.346 (1.072 to 5.134)	0.014

DISCUSSION

Bacterial vaginosis is one of the most common causes of abnormal vaginal discharge both in pregnant and non-pregnant women. The prevalence in this institution accounts to 70.57% non- pregnant and 67.4% pregnant patients seen at the outpatient department from 2017 to 2019. Despite the various reported cases of BV, still countless remain unrecognized. A previous systematic review showed diverse prevalence among populations of women, both low- and high- risk subsets of the general population, reporting widely heterogeneous estimates of prevalence globally, ranging from 5% to 58%.[8] Among pregnant women, prevalence worldwide ranges from 6 to 94%.[20]

The exact cause of BV is still unproven but believed to be due to alteration in the normal vaginal flora. It is likely that the normal flora may be overwhelmed by factors such as prolonged alteration in the pH of the vagina following vaginal douching and sexual intercourse. Alternative hypotheses contributing to BV include presence of multiple sexual partners and low socioeconomic status.[4]

The present study attempted to assess the risk factors associated with BV in the authors' institution. Women in their reproductive years are most likely to get BV, but can affect women of any age. In this study, the younger the age, the lower the risk to develop BV. Among women at 18 to 30 years old, a lower prevalence of BV was noted (50.18%) as compared to without (72.57%). Similarly, in a study done by Ranjit, et al.[13] the prevalence of BV was lower in younger women while higher among those at 30 to 40 years old. Likewise, Bhatarrai, et al. in Nepal, showed that the prevalence of BV was also highest among the age group of 31 to 40 years old and least among those below 20 years old.[21] The higher prevalence in the middle age group can be due to sexual practices, as they are considered to be a reproductively active age group.[13] Their age group has been reported to be commonly associated having more sexual partners and greater chance of engaging in unprotected sexual intercourse.[20] Furthermore, the middle age group was found to have greater variations in their menstrual cycle, like heavy and prolonged menstrual bleeding, that alters the vaginal ecosystem. Thereby, such factors may contribute to a higher risk of developing BV in the abovementioned age group. On the other hand, the presence of BV in the younger age group may be attributed to early sexual debut and changes in the sexual behavior of young people.

According to this study, more multigravid women have BV at 57.56% compared to nulligravid and primigravid women but the association was not statistically significant (p value 0.057). A higher prevalence of BV was also found in multiparous women (40.96%) while more nulliparous women have no BV (46.9%). This was similar to the study of Kamga, et al, wherein the highest prevalence of BV was seen in women with parity greater than four.[19] The possible rationale for this is that there may be a resulting disturbance in the vaginal microflora occurring during pregnancy and childbirth. If a woman becomes pregnant more frequently, such

predisposition to BV occurs repeatedly putting multiparous women at greater risk. In contrast to the present study, primiparity was more commonly associated with BV in the study conducted by Tachawatcharapunya, et al. in 2017.[22] In their study, primiparity may be associated with poor hygienic practices which they attributed to be more common in the younger age group.

Various studies have pointed the relationship between pregnancy and BV. It has been said that pregnant women are at increased risk due to hormonal changes during pregnancy. Estrogen tends to be protective against BV because it is known to increase glycogen that promotes lactic acid production making the vaginal pH acidic favoring colonization of lactobacilli in the vagina.[23] However, progesterone, which is present at greater concentration than estrogen in the first and second trimester, has been found to be negatively associated with glycogen levels. This is possibly the reason why among the 122 pregnant women with BV, the highest prevalence was found in the second trimester (18.45%) as compared to those in the first trimester (12.92%) and third trimester (13.65%). This was similar to the study by Kamga, et al., wherein the prevalence with respect to gestational age was highest during the second trimester.[20] However, in the present study, a significantly higher percentage of women having BV were in the non-pregnant 54.98%) compared to pregnant group (45.02 %). Although, according to several studies, the risk of BV is not dependent on the age of gestation. But with BV occurring in early pregnancy, risk of complications is greater compared to its occurrence in late pregnancy. In a study by Riduan, et al., it was concluded that only BV in the second trimester predisposes to a greater risk for preterm delivery.[24] Another study by Purwar, et al., found that BV diagnosed in the second trimester was associated with an increased risk of preterm labor, premature rupture of the membranes and subsequent prenatal birth.[17] The other complications of BV include miscarriage, chorioamnionitis, endometritis and poor neonatal outcome. These adverse effects have been reported to be due to presence of enzymes that reduce the ability of leukocytes prevent infections and presence of higher levels of interleukin-1 beta, an inflammatory cytokine, among pregnant women.[4] In contrast, in a study done by Achdiat, et al., BV is more likely to develop on the first and last trimesters of pregnancy.[18]

With regards to educational status, there was no statistical significance between the difference in the proportion of women with BV (p value 0.170). Among the 16 cases with no education or elementary undergraduate, there were 15 women who developed BV. In the study of Ranjit, et al.[13] illiterate women had the highest BV prevalence. Moreover, BV prevalence was also high in women who had no education in a study by Kamga, et al.[19] Low socioeconomic status, lack of education, hesitance to approach medical service and sociocultural structure might be the predisposing factors among less educated women who develop BV. Consequently, lesser level of education has been associated with low income status which affects hygiene practices that predispose to BV.[19]

Bacterial vaginosis was not significantly associated with coitarche or age of first sexual intercourse (p value 0.678). In the present study, comparable prevalence was seen in those with coitarche of less than 20 years old (52.77%) and in those 20 to 44 years old (47.23%). For the number of sexual partners, highest prevalence was seen in women with 2 or more partners at 68.63% which was significantly higher compared to the cases with one partner (p value < 0.001). Possible rationale for this is the imbalance in the vaginal microflora secondary to exposure to exogenous factors such as semen.[10] Seminal fluid is regarded to be alkaline in pH, hence, frequent exposure to semen may increase the vaginal pH that consequently reducing the concentration of Lactobacilli.[25] In turn, this favors the proliferation of BV-related organisms. Similar to that reported by Fethers, et al.[16] there is a strong and consistent association between BV and number of sexual partners. Although BV is not a sexually transmitted disease, the exposure to increased numbers of male sex partners or having recent change of partner increase the risk of acquiring BV. However, according to Koumans, et al, the risk of acquiring BV is greater among women having sex with women than those having sex with men. Moreso, in the same study, they reported a strong association between BV and history of women having female sexual partners. It is possible that the exchange of vaginal secretions between women during sexual intercourse predisposes a greater risk for BV.[3]

Furthermore, in this study, the authors found that non-contraceptive users have BV prevalence at 50.55% as compared to 49.95% among those cases using contraceptives. For those using family planning methods, the highest prevalence was found in women using condom at 20.66%. Several studies analyzed the effect of condom with varying protection

for BV. It has been proposed that condom acts as a barrier against bacterial cytotoxins which may play a role in lowering BV risk.[12] In the present study, the authors analyzed that the effect of condom have low protection for BV. However, among the 8 cases with IUD and the 4 cases who underwent BTL, all of them had BV. A positive relationship was seen with IUD use and BV. In a study done by Peebles, et al.[27] copper IUD users have elevated BV risk that persisted throughout use. The proposed mechanism is that the presence of IUD as foreign body in the uterus facilitates the overgrowth of anaerobic and facultative bacteria. In addition during the first 20 days from IUD insertion, the risk of infection is high since it can potentially facilitate the entry of organisms from the lower genital tract to the upper genital tract.[28] Lastly, only 9.96% of combined OCP and 9.59% of POP users had BV. This is due to the fact that the use of hormonal contraceptives were found to be protective for BV.

One explanation is the presence of estrogen stimulating the vaginal epithelial cells to produce more glycogen. This will create a more favorable environment for lactobacilli preventing colonization of anaerobes.[10] Aside from estrogen, progesterone is also known to thicken the cervical mucus that serves as a protective barrier that prevents organisms from the lower genital tract to ascend to the upper genital.[29]

The presence of comorbidities affecting the risk of BV was noted to be statistically significant (p value < 0.001). The highest prevalence was seen in cases with single or combination of co-morbidities. The highest was seen in those with presence of 2 or more co-morbidities followed by women with diabetes mellitus. Presence of comorbidities may impact the risk of concomitant infection such as bacterial vaginosis. In cases of diabetic women, the influence of glucose metabolism is seen in many infectious diseases, making them vulnerable to sepsis or other complications of bacterial invasion. Vaginal candidiasis is a more common lower genital tract infection associated with poorly controlled glycemia but several studies have also found its association with BV.[30]

Moreover, current study revealed that among women who were smokers, there were 32 out of 33 cases who had developed BV. There was statistically significant higher proportion of cases with BV among those who smoke (p value 0.001). Various studies hypothesized that the association between smoking and BV is due to the chemical contents of cigarette smoke which alter the vaginal microflora or may act by depleting the Langerhan cells in the cervical epithelium leading to local immunosuppression.[13] In a large observational study by Nelson, et al, smoking has been shown to be a dose-dependent risk factor for the diagnosis of BV, as well as with Trichomonas vaginalis, Herpes simplex virus type 2 and Chlamydia trachomatis. Smoking is linked with presence of damaged cervical epithelium through DNA modification and suppressed local and systemic immune responses. The major metabolite of Nicotine, Cotinine, has been shown to be concentrated in cervical mucus of smokers suggesting their relationship.[31]

Lastly, in this study, out of 53 cases of women who were alcoholic drinkers, about 45 of them developed BV. A statistically significant higher proportion of alcoholic beverage drinker had BV (p value 0.014). This finding was consistent with a study by Froele, et al.[32] which stated that alcohol consumption was associated with an increased risk of BV. The mechanism remains uncertain but maybe due to the depletion of hydrogen peroxide-producing lactobacilli as an effect of alcohol.[28] In addition, in another study by Smart, et al, higher quantities of alcohol of more than or equal to 140 grams per week is more likely associated with BV.[10]

The present study has several limitations, first, is the retrospective nature of the study wherein potential factors such as frequency of sexual intercourse, duration of menses, and length of use of contraceptive were not included since these are not usually recorded during consultations. Second, the study only determined general sexual behavior such as coitarche and number of sexual partners but did not reported other factors which may be contributory to BV such as douching or having partner with the same gender. The results of research may not be generalized to women seen in other institutions like government institutions wherein their subset of patients may have varying characteristics.

CONCLUSION

The prevalence of BV in this institution was found to be high at 70.57%. The high prevalence shows that it is a health concern among both pregnant and non-pregnant women. In conclusion, age particularly those in the older or middle age group, multiparity, pregnancy, multiple sexual partners, IUD and condom use, diabetic, smokers and alcoholic beverage drinkers have less protection for BV. Present study findings

may be beneficial for preventive strategies for BV taking into consideration its occurrence rate and its association with various obstetrics and gynecologic complications.

RECOMMENDATION

A prospective study design with equal number of subjects is recommended to have a more favorable comparison between the risk factors and bacterial vaginosis. Future studies may include history of previous sexually transmitted infections or previous BV infection who underwent treatment. They may also include risk factors leading to BV recurrence. These will aid future studies to a more enhanced diagnosis and management of BV.

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