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Determining the Predictors of Adverse Pregnancy Outcomes in Females Presenting with the Complain of Vaginal Discharge

Amna Tariq

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ABSTRACT

Background: Bacterial vaginosis (BV) is an important genital syndrome affecting a large number of the women in the reproductive age group. It is identified by alterations in the normal vaginal flora and a malodorous discharge when symptomatic. Some of the risk factors for BV include low socio-economic status, poor hygiene, cigarette smoking, douching, antibiotic use for other conditions, young age of coitarche, new sex partners or multiple sex partners, race, ethnicity, education, income and age. This study will be determining the correlation of BV with the adverse pregnancy outcomes and the predictors that affect the adverse pregnancy outcomes in women diagnosed with bacterial vaginosis (BV).

Methods: Secondary data set from Rawalpindi Pakistan was analyzed using SAS. Outcome parameters included abortions, ectopic pregnancy and still birth. Chi-square test (for numbers) was used for bivariate analysis to determine the significance of association between categorical variables. The t-test was used for the univariate analysis of numeric variables. The potential risk factors, associated with bacterial vaginosis from the literature and results of this study were evaluated by logistic regression analysis. A multivariate logistic regression was run to look at all the adverse pregnancy outcomes of Bacterial Vaginosis individually and as a combination by controlling for all the socio-demographic and sexual risk factors.

Results: About half (49.4%) of the women had an adverse pregnancy outcome in the past, and abortion was the most prevalent (37.7%). A history still birth was seen in 31(9.3%) females and ectopic pregnancy was also observed in 31 (9.3%) females. On bivariate analyses, educational status (P-value= 0.0026), Economic status (P- value= 0.0010), having

alive children (P-value=0.0001) and pregnancy status of women (P-value= 0.0327) showed a statistically significant association with the adverse pregnancy outcomes. Multivariable analyses showed no statistically significant associations between BV and the aggregate adverse pregnancy outcomes. Among the population characteristics, economic status and having an alive child showed significant associations with the adverse pregnancy outcomes. Specific outcome analyses showed no significant association between BV and abortions, still birth or ectopic pregnancy.

Discussion: For this study adverse outcomes were studied as individual entities, abortions, still birth and ectopic pregnancy and also as a combined entity (presence of either of the mentioned adverse outcome). We tried to look at the association of the history of past pregnancy outcomes and current diagnosis of BV in women at the time the study took place. In this study **i**ncreased odds for abortions (OR=1.210, CI:0.506,4.451) were seen in people with bacterial vaginosis compared to the people without bacterial vaginosis when diagnosed with BV on the basis of Nugent scoring system, the gold standard criteria. Overall, this study revealed a mixed result of BV having and association with adverse pregnancy outcomes in females, based on any of the diagnosing criteria. Economic status was found to be significantly associated with the adverse pregnancy outcomes in females diagnosed with BV.

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Author's Statement Page

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1.0 INTRODUCTION

1.1 Background

Bacterial vaginosis (BV) is an important genital syndrome affecting a large number of the women in the reproductive age group (Morris et al. 2001; Sumati and Saritha 2009). It is identified by alterations in the normal vaginal flora and a malodorous discharge when symptomatic. Vaginal discharge is a common vaginal symptom that encourages the women to seek medical care. (Sobel 1997). BV is the most prevalent and the least understood problem among the women of child bearing age group (Ness et al. 2002). It is one of the most important causes of vaginal discharge. Multiple demographic and behavioral factors have been associated with the occurrence of BV (Rein and Holmes 1983; Cherpes et al. 2008; Fethers et al. 2008). The female vagina is a complex environment containing variable amount and proportion of bacterial species. The normal vaginal flora is maintained by a complex balance of various microorganisms. The actual processes and microbiological events, leading to a shift in the normal vaginal flora causing BV is still not well understood. BV appears as an asymptomatic condition in the majority of the females (Schwebke et al.1997). BV is considered one of the most common causes of vaginitis. BV has emerged as a global issue in the recent years due to its association with ascending genital tract infections and sexually transmitted diseases (Kouman and Kendrick 2001; Nyirjesy 2008; Verstralen et al. 2010; Kumar et al. 2011). Multiple risk factors have been associated with BV which include a low socio-economic status, poor hygienic conditions of women, cigarette smoking, regular douching, use of antibiotics for other conditions, young age at the time of first sexual activity, new sex partners or having multiple sex partners at the same time (Verstraelen 2008; Fethers et al. 2009; Verstraelen et al. 2010). Race, ethnicity, educational status, income level and age

of women are significant correlates of BV affecting its prevalence and incidence in a population (Allsworth and Peipert 2007).

1.2 Research Question

This study will determine the correlation of BV with the adverse pregnancy outcomes and the predictors that affect the adverse pregnancy outcomes in women diagnosed with bacterial vaginosis(BV). In this study, adverse pregnancy outcomes of bacterial vaginosis include spontaneous abortions, still birth and ectopic pregnancy. In this cohort of individual's, all the women presented with the complain of vaginal discharge. These women were either diagnosed with BV or any other conditions like vaginitis, pelvic inflammatory disease and/or other sexually transmitted diseases like Chlamydia or Gonorrhea. Patients with BV generally complain about different combination of vaginal discharge, odor, irritation or itch which differentiates them from the patients with vaginitis. In BV discharge is characterized by its particular color (white), consistency (homogenous), odor(fishy) and amount which cannot be quantified (Anderson et al. 2004). The term "vaginosis" is used instead of "vaginitis" for BV, since it is often not associated with the clinical signs of inflammation as in cases of vaginitis or PID (Mashburn 2006).

1.3 Adverse Pregnancy Outcomes and predictors of BV

Studies have shown than an association between BV/vaginitis and its associated intrauterine infections can result in gynecological complications like spontaneous abortions and preterm birth (Gravett et al. 1986; Hillier et al. 1995; Thorsen et al. 2006). The association between BV and intrauterine infections is also responsible for approximately 70% of the neonatal deaths. Newborns also suffer from long-term neurologic morbidity due to this association (Hack 2000; Benedetto et al. 2004). BV causing microorganisms and the toxins

produced by these microorganisms are capable of crossing the placenta and resulting in brain injury in the fetuses. This ultimately results in fetal morbidity. In women with a previous history of having suffered a preterm birth or with a history of low pre-pregnancy body weight, treatment of BV has shown to significantly decrease the rates of preterm birth, preterm labor, low birth weight babies, and premature rupture of membranes (Mikamo et al. 1999; Rezeberga et al. 2008). BV in particular is associated with increased gynecological complications, such as low birth weight babies, premature rupture of the membranes, preterm birth, preterm labor, miscarriage, spontaneous abortions, intra-amniotic infections, postpartum maternal infections, chorioamnionitis, and infertility. Other gynecological complications such as post-operative infections (hysterectomy, legal abortion) have also been seen to occur due to BV (Larsson et al. 1989; Leitich et al. 2003; Benedetto et al. 2004; Larsson et al., 2005). The method currently employed for the diagnosis of BV is the assessment of clinical signs, but the clinical signs can be misleading and their detection relies on the expertise of the clinician performing the clinical exam (Nugent et al. 1991). Hence, the diagnosis of BV is made on the basis of both, clinical examination and the laboratory signs, which includes the Amsel criteria and the Nugent scoring system.

Some of the predictors that will be looked at in the study, affecting the adverse pregnancy outcomes in women include age of the women, age at which the women became sexually active, economic status of women, educational level, frequency of changing pads during the menstrual cycle, pregnancy status of women at the time the study was conducted and the number of alive children she had till the time she presented to the hospital with the complain of vaginal discharge. BV has a strong association with age since BV has been found to be more common among females over 25 years in comparison to the STIs which are found more commonly in

women younger than 25 years of age (Sewankambo et al. 1997; Morris 2001; Wilson et al. 2002).

BV is considered as one of the most commonly occurring vaginal disorder in females of the reproductive age group. BV is a frequently occurring condition across different populations around the world as can be seen in the literature review. Abnormal vaginal flora has been related to the adverse pregnancy outcomes in women. Pregnancy outcomes and the prevalence of adverse pregnancy outcomes differs in women suffering from BV when compared to women suffering from any other vaginal condition like PID, vaginitis and cervicitis. Demographic and socioeconomic factors associated with bacterial vaginosis impact the pregnancy outcomes in females. Therefore, correlating these factors to the adverse pregnancy outcomes will emphasize on the importance of socioeconomic and demographic factors that can affect the pregnancy outcomes, especially in the developing countries where illiteracy and poverty prevails. This study will also lead us to conclude if bacterial vaginosis is a greater risk factor for adverse pregnancy outcomes in comparison to other conditions associated with abnormal vaginal discharge. This will allow the physicians to be vigilant while treating a pregnant women or an infertile women diagnosed with bacterial vaginosis.

2.0 LIETRATURE REVIEW

The microbiome of the female vagina impacts the overall health of women, their pregnancy outcomes, and the health and wellness of the neonates (Marrazzo et al. 2010). The microbiota of the healthy lower genital tract in women predominantly consists of Lactobacillus spp., with L. *crispatus*, L. *jensenii* and L. *iners* being the most prevalent species. (Turovskiy et al. 2011) These species of bacteria protect the vagina by forming a critical line of defense against

the genital pathogens. A dynamic vaginal ecosystem exists in the vagina where the hormones circulating in the female's body modulate the symbiotic relationship between the vaginal epithelia, vaginal lactobacilli, vaginal secretions and the innate immunity factors (Marrazzo et al. 2010, Turovskiy et al. 2011).

Bacterial Vaginosis is an enigmatic condition, a poly-microbial syndrome whose etiology and pathogenesis is still unknown (Fethers et al. 2012). BV is the most common globally occurring vaginal infection affecting the women of child bearing age group, and is correlated with adverse pregnancy outcomes, an increase in heterosexual HIV transmission and the transmission of other STDS (Turovskiy et al. 2011; Fethers et al. 2012; Gillet et al.2011). The pregnancy outcomes correlated to BV include an increased risk of late miscarriage, low birth weight babies, preterm labor, post-partum endometritis, chorioamnionitis and post-abortion pelvic inflammatory diseases (Demirezen). BV is the most prevalent reason for the vaginal discharge and hence for the women to consult for medical help (Gillet 2011). BV is also one of the commonest causes of vaginitis along with vulvovaginitis candidiasis (Koumans and Kendrick 2001; Nyirjesy 2008; Verstraelen et al. 2010; Pal K et al. 2011).

In the normal vagina, lactobacilli species protect the vaginal flora from the potential pathogens by producing lactic acid, H_2O_2 and the antimicrobial proteins (Demirezen). This results in healthy pregnancies, healthy newborns, a reduced risk for many sexually transmitted pathogens and lack of abnormal vaginal symptoms (Edwards 2004). Bacterial vaginosis, is a poly-microbial syndrome described by the replacement of lactobacilli with other anaerobic microorganisms such as *Gardnerella vaginalis* and *Prevotella*, *Peptostreptococcus* and *Bacteroides* spp which continue to flourish unrestrained in the vaginal fluid, hence increasing in numbers immensely, resulting in a loss of the protection of the vagina (Turovskiy et al. 2011).

Hence the normal vaginal ecosystem gets disturbed resulting in vaginal infections, vaginal discharge and the transmission of STDs (Schwebke 2005; Hampton 2006; Atashili et al. 2008).

According to the Centers for Disease Control and Prevention (CDC), infections related to BV can be classified broadly as opportunistic infections. BV can occur and coexist with other infections that occur due to sexually transmitted agents/bacteria (Koumans, Kendrick ,2001). In 1921 Schröder, classified the vaginal discharge into three different types. The first type of discharge was dominated by Lactobacilli species, the second type of discharge was a combination of Lactobacilli species and other bacteria, and in the third type of vaginal discharge no Lactobacilli bacteria were present. The term Bacterial Vaginosis was coined in 1984 and defined as the replacement of vaginal lactobacilli with bacteria that cause a change in the characteristics of the normal vaginal fluid. (Hillier et.al, 1996)

BV is a very commonly occurring vaginal disorder in females of the reproductive age range. The frequency of BV has been pretty high, ranging from 3.6-40% as observed in different populations around the globe (Forsum et al. 2005). Majority of the studies related to BV have been conducted in clinics like genitourinary medicine clinics (GUM), STI clinics, abortion clinics, and primary care units of hospitals (Larsson et al. 2005). These studies included multiple study populations that were selected from the STI clinic or the gynecology and obstetrics clinics. Research on BV in the healthy female population is hard to find. There is a lack of literature on BV in the population based studies in the healthy female populations (Morris et al. 2005; Allsworth, et al. 2008). BV related research studies have been conducted on different categories of female populations including pregnant females, females presenting at the clinics for abortions, patients with a complain of vaginal discharge and sex workers presenting with vaginal complaints (Larsson et al. 2005). BV has been seen to be particularly predominant in the African

region. Multiple studies have reported high prevalence rates of BV in Africa. In Africa BV has been found in about 20-49% of the females attending the STI clinic, 21-52% in pregnant women attending the antenatal clinics, and 37-51% of BV has been found in females who participated in the community based studies. These rates are greater than those recorded in the industrialized countries: 13% in GUM clinics in the United Kingdom, 11% in gynecology clinics in UK and 15-30% in the women in USA who are non-pregnant (Ledru et al. 1996; Govender et al. 1996; Thomas et al.1996). Currently, there are no studies available to support the changes in vaginal flora that occur among pregnant women during gestation or the prevalence of BV during gestation (Nelson 2002).

There are many risk factors for BV which include demographic, hygienic and sexual factors as will be discussed later in the section (Hillier et al. 1996; Verstraele et al.2010; Allsworth et al. 2005). High risk sexual behaviors are a risk factor for acquiring sexual diseases and it suggests that Bacterial Vaginosis can also be transmitted sexually. A typical sexually transmitted infection usually involves a single etiological agent, however in case of BV, multiple pathogens are seen in excessive numbers. Low numbers of the same pathogens are identified in the vaginas of women who are not infected with BV and/or have never been sexually active (Verstraele et al. 2010).

2.1 Prevalence

Bacterial Vaginosis is one of the most commonly occurring vaginal disorders. Studies have shown a high frequency of BV among different populations around the world (Forsum et.al, 2005). The prevalence of BV in asymptomatic and pregnant women have been seen to be 12-25 % each (Nejad V, Shafaie S.,2008). Studies on BV in healthy female population are difficult to find (Morris et al., 2001, Allsworth et al., 2008). BV has been found to be common in Africa.

The rates for BV are much higher in the developing countries when compared with the developed part of the world (Ledru et al.1996; Govender et al. 1996; Thomas et al. 1996; Morris et al. 2001; Holzman et al. 2001). In Uganda a population based survey took place to report the prevalence of BV which came out to be 50% (Wawer et al. 1999). Since this figure is a particular characteristic of rural African area which lacks the education about basic hygiene practices, we cannot make this generalizable and apply it to other settings. But we can conclude that the prevalence rates are visibly different in the developing countries when compared to the developed countries.

American and European studies have reported a prevalence of BV between 4.9 to 36%. The first nationally representative study on BV in the United States was conducted in 2001 by National Health and Nutrition Examination Survey, NHANES. It was conducted according to the different races and ethnicities in the US. The results depicted higher rates of BV in African American women (50.3%) and Mexican American women (28.8%) compared to the white women (22.4%) (Hampton et al. 2006; Allsworth et al. 2007). BV is approximately two to three times more prevalent among the black women as compared to women of white color (Kouman et al. 2007). Sexual orientation is also an important predictor of BV. Higher infection rates of BV, ranging between 29% and 52% have been seen among the lesbian populations (Schmid et al. 1999). Approximately 33% of the young girls without any history of sexual activity have shown to have BV in their vaginal microbiota (Shafar et al. 1989). Depending on the population being studied, the overall prevalence of BV varies greatly. In the general population, among the women in the child bearing age bracket, the prevalence of BV varies from 10% to 25%, which is significantly high (Nansel et al., 2006).

2.2 Risk factors

Multiple risk factors have been associated with BV. These risk factors can be broadly classified into demographic risk factors and the sexual risk factors. Some of the significant demographic correlates for BV include socio-economic status, income, educational level of women, race, ethnicity, smoking, higher body mass index and age (Forcey et al. 2015; Yzeiraj-Kalemaj et al. 2013; T.Ashraf-Ganjoel et al. 2017). Women belonging to the age group of more than 25 years, with black ethnicity and lower socio-economic status have been strongly associated with bacterial vaginosis (Morris et.al 2001). Use of contraceptive pills, frequency of sexual intercourse, non-circumcised male sexual partner, vaginal douching and female to female sexual contact are other risk factors associated with BV. BV is seen to be prevalent in populations that have shown high rates of sexually transmitted infections and increase in risky sexual behaviors like sexual debut at a young age of under 25, frequently changing or multiple sexual partners and lack of condom use during a sexual activity. Sexual practices like anal sex, sex toy use and ROS have been considered to contribute to the development of BV. BV also increases the risk of the females to acquire HIV and other STIs. (Fethers et al., 2009; Forcey et al. 2015; Morris 2001). Consistent use of condom during a sexual activity has been found to be protective against the development of BV. Reducing the number of active sexual partners and decreasing the frequency of unprotected sexual encounters also decreases the risk for incident and recurrent BV. Recurrence of BV is seen in sexually active couple even after the use of antibiotic treatment (Fethers KCAA, Fairley CK, Hocking JS, Gurrin LC, Bradshaw CS.,2008).

2.3 Microbiology of BV

The microbiota of a healthy female vagina undergoes many changes throughout lifetime. The presence of natural bacteria in the vaginal environment is a balance of many factors

including the host's genetic predispositions, vaginal environment and sexual behavior. The complex bacterial environment is maintained as a result of the circulating estrogen in the females (Turovskiy et al. 2011). In normal circumstances lactobacilli comprise of 95% of the bacteria in the vagina. These bacteria produce abundant antimicrobial compounds including lactic acid and H_2O_2, hence protecting the vagina against the growth of multiple pathogens by creating an acidic environment. Lactobacilli also produce biofilms that fend off the pathogens. In BV, lactobacilli are severely reduced or absent from the vagina resulting in an overgrowth of anaerobic bacteria including *Gardnerella vaginalis, Ureaplasma urealyticum, Mycoplasma hominis, Mobiluncus* species and *Prevotella* species and other anaerobes. Sometimes the number of these bacteria grow 100-1000 fold above the normal bacterial counts in the female vagina, causing symptoms of BV (Linhares et al. 2011; Afolabi et al. 2016; Turovskiy et al. 2011).

2.4 Physiology of BV

In bacterial vaginosis, the normal lactobacilli friendly vaginal environment gets replaced by anaerobic microorganisms like *G. vaginalis, Prevotella, Peptostreptococcus and Bacteriodes* spp. Other lesser known species of bacteria like *Atopobium vaginae, Clostridium* spp, *Leptotrichia* spp, and *Megaspharea* spp have also been associated with BV by many different studies. Historically *G. vaginalis* has been considered as the leading cause of infection making the vagina a suitable environment for the anaerobes to thrive and cause the vaginal symptoms. *G. vaginalis* belongs to the genus *Gardenerella. G.vaginalis* is described as a gram-uncertain organism because its gram stain result can vary from being gram positive to gram negative. When a pure culture of *G. vaginalis* is grown on a medium containing starch, it appears as gram variable. It shows that the age of the culture, growth conditions and the thickness of the peptidoglycan cell wall layer affect the reaction that the bacteria shows towards gram stain. The

cell wall of *G.vaginalis* is multilayered, low in peptidoglycan content (20%). The cell-wall lacks ribitol and techoic acid. The cells of *G.vaginalis* are generally small, pleomorphic rods measuring 0.4 by 1.0-1.5 micrometers with minute variations. The bacteria is immobile, contains a small genome (1.62-1.67 Mb), lacks important enzymes in biochemical pathways that synthesizes amino acids, thus it lives as a parasite. Biochemical tests have shown *G.vaginalis* to be catalase positive, oxidase and β-glucosidase negative. The idea of the mere presence of anaerobic organisms causing BV has been challenged and attributed to the fact that unrestrained increase in the number of these organisms reaching 10-1000 fold above the normal are also a major contributor to the development of BV (Turovskiy et al. 2011).

2.5 Vaginal conditions and complaints

Vaginal complaints in the form of vaginal discharge or discomfort are the one of the most common reasons for the women to visit primary care hospitals for the gynecological examination and account for approximately 10 million visits per year. The most commonly detected vaginal conditions are candidiasis, bacterial vaginosis and trichomoniasis, which are the commonest reasons of vaginitis, that occurs most commonly in the reproductive age group. The prevalence of these three conditions varies depending on the clinical settings and the areas. According to national figures, 40-50% of people who have vaginal discharge are diagnosed with BV, 20-25% are diagnosed with vaginal candidiasis and 15-20% have trichomoniasis. A large number of women remain undiagnosed. Other uncommon reasons for vulvovaginal complaints include infection with herpes simplex, mechanical irritation in the vagina due to lack of lubrication, allergic reactions to chemical irritants, latex or semen and atrophic vaginitis in post-menopausal women. It is estimated that approximately 30% of the women remain undiagnosed with the

vaginal complaints even after a comprehensive examination. Some of the complaints often associated with vaginitis are discharge, odor, irritation or itch. Discharge can be clear, white, green, yellow or gray in color; thick, thin or curd like in consistency; and the amount can be more or less than the usual. The irritation manifests as either erythema, excoriation or discharge on the perineum or intriotus (Anderson et al. 2004).

BV has a strong association with pelvic inflammatory diseases, cervicitis and upper genital tract infection (Ganjoel 2005). Chlamydia trachomatis, Neisseria gonorrhoeae, T. vaginalis, HSV, and HPV are frequently associated as the causative organisms for cervicitis, another common vaginal condition found in women (Holmes 1999). This condition is painless and it becomes obvious only by the presence opaque yellow colored, mucoid to watery discharge, resulting in bleeding on impact or contact and deep dyspareunia. Cervix also appears to be red in color and swollen with erythema, ectropion and inflammation (Petersen, 2006). Ascending infection from lower to the upper genital tract leads to pelvic inflammatory disease (PID) (Stanbery and Bernstein 2000). PID can result in conditions like pelvic peritonitis, endometritis, salpingitis, tubo-ovarian abscess, or a combination of the mentioned conditions (Holmes 1999). The most commonly associated causes of Pelvic inflammatory diseases are N. gonorrhoeae, C. trachomatis, *G. vaginalis, Prevotella, Peptostreptococcus and Bacteriodes* spp and the Streptococci spp. (Ness et al. 2004; Petersen 2006)

2.6 Hygiene practices

Behavioral practices such as persistent vaginal douching and menstrual hygiene practices have been associated with the change in the vaginal flora (Fethers et al. 2009). Douching has been seen to be associated with abnormal vaginal flora (Forcey et al. 2015). Douching is more common among the African American women in the United Kingdom and has been linked to dry

intercourse which results in an increase in the bacterial lower genital tract infections. Douching is considered as an independent risk factor for BV (Morris 2001). Some of the studies pointed out menstrual, personal and coital hygiene as the hygiene associated cofactors related to BV. A correlation was observed between different methods of contraception and BV. BV was seen to be significantly present in the female populations who used intrauterine contraceptive devices persistently (Bahram et al., 2009). Some studies have shown that less than 14 days since onset of menses was strongly associated with the incident BV but not prevalent BV (Forcey et al. 2015). There is a need to further investigate the association between the vaginal cleaning practices and BV (Morris MC. 2001**).**

2.7 Concurrent infections

BV has been associated with concurrent chlamydial and gonococcal genital infection but not with the risk of development of these infections. A stronger association has been observed for anaerobic gram negative rods (Ness et al. 2005). An association between HSV-1 and HSV-2 and BV has also been observed, although the risk of concurrent HSV-2 infection is higher than HSV-1 infection. BV has seen to increase the chances of acquiring HSV-2 in comparison to the women with normal vaginal flora. Studies have also indicated a correlation between HIV infection and HIV shedding with BV. BV also increases the risk of HPV infection (Allsworth et al. 2008).

2.8 Complications of BV

BV has been associated with adverse obstetrics outcomes such as abortion, preterm labor, premature delivery, premature rupture of membranes and miscarriage (Demirezen 2016; Giakoumelou et al. 2015). BV is a strong risk factor for the mid-trimester miscarriage. BV in

early pregnancy has been strongly associated with short term adverse effects leading to a late miscarriage. Not as strong an association has been seen between BV and perinatal mortality and neonatal infection (Leitich and Kiss 2007). A statistically significant association is found between the history of abortions and presence of BV. A two to three- fold higher risk of spontaneous abortions was seen in women suffering from BV as compared to the women who did not suffer from BV. It is postulated that inflammatory reactions following ascending infections due to BV can lead to spontaneous abortions (Nejad and Shafaie 2008).

In a recent Cochrane review, a decreased risk of miscarriage was seen in women who had taken antibiotic treatment for BV (Giakoumelou et al.2015). No significant association has been seen between BV and a history of recurrent pregnancy losses. BV is seen to be present more frequently in women with a history of spontaneous abortions in the last six months compared to women with recurrent pregnancy losses (Demirezen 2016). BV has been shown to be an important risk factor for preterm labor and chorioamnionitis. BV has an influence on the outcome of the labor activity and is considered to induce pre-term birth. Diagnosis of BV in the second trimester increases the risk for the early rupture of membranes and accounts for approximately 82.5% of the attributable risk for pre term birth. (Yzeiraj et al. 2013). The exact mechanism of BV inducing preterm labor is not yet known, however, it is said that anaerobic vaginal microorganisms like *Gardnerella vaginalis, Mycoplasma homonis, Peptostreptococus* and *Bacteriodes* replace the protective vaginal lactobacilli. The products of anaerobic bacteria stimulate deciduas and result in an increase in cytokines phospholipase A2 and prostaglandin release causing preterm labor ((Nejad and Shafaie 2008). Some studies have shown that a lactobacilli poor vaginal microbiota environment during pregnancy leads to shorter gestation and increase in pre-term birth (Ness et al. 2005). BV causes a majority of other serious gynecological

complications like postpartum and post-abortion endometritis, intra amniotic infections and pelvic infection (Turovskiy et al. 2011).

2.9 Diagnosis of BV

A combination of clinical signs and laboratory tests are used to diagnose BV. Laboratory methods for the identification of BV/vaginitis include performing a wet mount or gram stain, considered as the "Gold Standard" measure of diagnosis and the culturing of the microorganism for the confirmation of the diagnosis (Mehdinejad et al. 2011). Originally the method for diagnosis of BV was culturing the vaginal specimens for *G.vaginalis*. Other methods for the diagnosis of BV in the recent past included FemExam or BV Blue test. These tests are costly and non-specific. The first system for the diagnosis of BV was the Spiegel system that included grading of the microbial flora seen in the gram stained slides (Mohanty et al. 2010).

The most common diagnostic method currently utilized is the assessment of clinical signs. However, clinical signs are misleading and subtle and their detection depends on the expertise and experience of the clinician performing the clinical exam. This method is known as the Amsel criteria (Nugent et al. 1991). The Amsel criteria is based on the observation of the following signs that include the consistency of vaginal discharge: number of clue cells on the gram stained slide, odor of the vaginal discharge and the pH of the vaginal discharge (Amsel et al., 1983). Amsel criteria may not be enough when used to diagnose patients with BV as 50% of the patients may be asymptomatic. Moreover, there exists an intermediate category of bacterial vaginosis in which the lactobacilli species still predominate, and the Amsel's criteria is unable to detect it. The gram stain laboratory method, using Nugent scoring criteria is the least expensive diagnostic method. It also requires less time and is therefore the most widely used method for the detection of BV. This method quantifies the gram positive rods (Lactobacillus morphotypes),

gram negative and gram variable rods (Gardnerella sp. and Bacteriodes sp.), and curved gram negative rods (Mobiluncus sp.), and then records a score for them to detect bacterial vaginosis (Mohanty et al. 2010).

2.10 Treatment of BV

BV is traditionally treated with metronidazole and clindamycin and this has been recommended by the Centers for Disease Control and Prevention. However, this treatment does not eradicate the BV associated bacteria from the vagina in entirety and a 30-40% chance of recurrence remains. After the treatment, many women remain colonized with *G.vaginalis* and or other anaerobic bacteria and sometimes lactobacilli fails to rise back to the levels that are needed to protect the vagina from the anaerobic bacteria. Limited understanding of the etiology and pathogenesis of BV hinders a completely effective treatment and prophylaxis approach for BV eradication (Turovskiy et al. 2011; Nelson et al. 2016). However, some trails have shown a significant reduction in the rate of premature births among pregnant women who presented with a high risk of prematurity and had been treated with metronidazole in the past (Nejad and Shafaiq 2008). The chances of recurrence of BV one month after the treatment are also very common (Koumans et al. 2007).

3.0 METHOD AND MATERIALS

This study was carried out on a secondary data set. The primary data set was collected by Dr. Shireen Rafiq at Holy Family Hospital (HFH) Rawalpindi and Quaid-i- Azam (QAU) University Islamabad. Holy Family Hospital is a tertiary care teaching Hospital working in association with Rawalpindi Medical College, Rawalpindi, Pakistan. It serves a large population

of the Rawalpindi area, and is a major health care facility in the public sector of Rawalpindi region and its surroundings. Patient selection and sample collection for the study were completed over a time period of one and a half years between May 2009 and November 2010. Patients were selected from the out-patient department of the Gynecology and Obstetrics unit in Holy Family Hospital Rawalpindi. Selection of the female patients was dependent on the presentation and history of vaginal discharge. Three main methods were used for data collection for the purpose of this study. These included conducting a survey questionnaire. The questionnaire encompassed a complete history of each female patient including the sociodemographic information (including the age, education status, economic status), duration of vaginal discharge, symptoms and associated features, treatment history for the vaginal discharge, sexual history and its details, any history of pregnancy, pregnancy loss and treatment taken for it, history about husband's income and occupation and information around his sexual life. A detailed gynecological examination was perfumed which included a thorough assessment of genital tract infection. Inspection of genitals, per speculum examination of vaginal cavity and cervix along with the collection of vaginal samples for the purpose of laboratory diagnosis was done.

Patients were selected for the study based on the vaginal symptoms. The most common reason for the study population to show up for the gynecological consultation was the vaginal symptoms. The diagnosis of vaginal discharge was based on history, examination and diagnostic tests.

Additional **inclusion criteria** were:

- Females with complain of vaginal discharge
- Married and sexually active females
- Patients in the age range of 15 to 42 years

- Negative history for any use of antibiotic in the recent past
- Females who had not menstruated for 48 hours before their physical examination

Exclusion criteria were:

- Females with any surgical procedure performed on uterus
- Females on any antibiotic therapy or had taken antibiotic in the last two weeks
- Presently pregnant females, post-delivery and post abortion females
- Female subjects menstruating or bleeding per vaginal at the time of study
- Females with age above 42 years

3.1 History

Patient was asked about itching or rash over the perineum along with any changes in the color of the perineum, color and consistency of the vaginal discharge. The patient was also asked about the inter-menstrual bleeding, menstrual cycle, any unwell feeling during the menstrual cycle, complain of pain during intercourse, or spotting after having an intercourse. A comprehensive gynecological history was obtained from the patient regarding number of times she has conceived, the number of live births and pregnancy loss. The patient was inquired about the type of pregnancy loss like miscarriage, abortions, still birth and ectopic pregnancies. Patients were inquired about their monthly income level and the social status (low, middle and high) based on their monthly earnings. Since the study was conducted in a public sector hospital that drains majority of the low income populations, therefore, majority of the population fell in the low socio-economic strata. Literacy level of the patient was ascertained by asking them about their level of education and if they can read and write. Vaginal infections are affected by the economic and educational level of the patient as will be discussed further in the thesis.

3.2 Normal vaginal discharge

The color of normal vaginal discharge is clear to white with a slight variation in the consistency of the during different phases of the menstrual cycle. Consistency can vary from thin, to viscous and sticky in the mid cycle. There is no smell in the normal vaginal discharge, and its considered odorless.

3.3 Elicitation of symptoms

Patients who had vaginal infection generally presented with the complain of vaginal discharge having some color, odor and consistency, which was contradictory to the normal appearance of discharge. They also complained of some irritation or itching in the perineal area. The color of the discharge was either white, yellow, gray, green or clear. Consistency of the discharge was found to be homogenous, watery, thick, or curd like. Odor was foul, pungent, or fishy. There was no scale available to quantify the amount of vaginal discharge.

3.4 Physical Examination

Internal and external physical examination was performed. For the purpose of conducting the vaginal examination patients were asked to lie on the examination couch in the lithotomy position with abundant light falling on the pelvic area. A dry cuscos speculum without any lubricant or antiseptic was inserted into the vagina. Condition of vulva and perineum were examined for any signs of rash externally. Internal vaginal condition was assessed with the help of speculum.

3.5 Specimen sampling and preparation

The presenting symptoms of vaginal discharge are usually localized in the vaginal area and endocervix. For the patients selected on the basis of history and clinical signs, high vaginal swabs (HVS), endocervial swabs and blood samples were obtained. Vaginal and endocervical samples were taken with the help of cotton swabs and cotton swab with Amies transport media (Citotest Transport Swab; Amies with Charcoal GAMMA Sterile; Biomed). Four samples were obtained in total. Two high vaginal swabs with sterilized cotton swabs and two endocervical swabs in Amies transport media were taken by rotating the swab in counter clockwise direction. After obtaining the samples the speculum was removed, and pH (pH indicator strip; Merck, pH ranges 3.8-7.4) of the vaginal discharge was determined. Potassium hydroxide (KOH) was poured on any discharge present on the speculum for the detection of Amine odor (Whiff test), a confirmatory test for BV based on Amsel criteria.

Each swab was carefully labeled with patient's serial number, patients name and the date of the collection of sample. Endocervical swabs were collected with extreme care to avoid any sort of swab contamination that could occur on contact with the vaginal wall and vaginal discharge. Out of the four swabs obtained, first swab, each of vagina and endocervix was used for Giemsa staining and direct smear gram staining. The second one was used for inoculation on the media plates. A blood sample was drawn for female patients who agreed to be investigated for Chlamydia trachomatis (STI). The blood sample was drawn under aseptic measures from the cubital vein. The blood was centrifuged at 3000 revolutions per minute and serum was separated in the eppendorf. After properly labeling the test tube with name and number, serum was stored at -35ºC to keep it viable.

3.6 Gram staining

Gram staining of the direct smear was performed for the confirmation of the diagnosis of Bacterial Vaginosis according to Amsel Clinical Criteria and Nugent scoring system. Gram staining was carried out after obtaining the isolates from the growth on media plates for the identification of the isolates. Gram stains required for performing the gram staining procedure were prepared according to the instructions in Koneman's (Konemans, 2006; Karamat, 2012).

3.7 Amsel criteria

The characteristics of normal vaginal discharge are composed of clear to white in color, floccular, odorless, and thin and viscous in consistency. In clinical practice Amsel clinical criteria is the most commonly used measure for diagnosing BV. It is considered a gold standard for the clinical diagnosis of bacterial vaginosis. Amsel criteria was applied on all the patients selected for this study to determine the presence of BV.

The diagnosis was positive if any three out of the four following criteria were fulfilled.

1) Presence of thick, white, homogenous and smooth discharge

2) The vaginal fluid had a pH > 4.5 (determined by the pH indicator strip; Merck, pH ranges 3.8-7.4)

3) Whiff test, Sniff test or the Amine odor test: Vaginal discharge gave off fishy odor before or after addition of 10% of potassium hydroxide (KOH).

4) There were clue cells present, when visualized on gram stained microscopic examination.

Presence of any three of the above four criteria makes the BV diagnosis confirmatory.

3.8 Nugent scoring system

Nugent scoring is the most frequently used authentic and standardized laboratory procedure for diagnosing BV. Each gram stained direct smear obtained for each patient was evaluated for the Nugent scoring (1991) under oil immersion lens (x1000 magnification). Morphotypes were scored as the average number of bacteria seen per oil immersion field. Total score was given by adding the individual scores of lactobacilli,G. vaginalis and Mobiluncus. Each bacterial morphotype was individually quantitated from a score of 1 to 4+ with regard to the number of their mophotypes seen per oil immersion field.

0, no morphotype

1+, less than 1 morphotype

2+, 1-4 morphotypes

3+, 5-30 morphotypes

4+, 30 or more morphotypes.

The amount of bacteria (G. vaginalis) present in the sample was also rated on a point system (from 0 to 4 points), but the points were assigned in the opposite way. The presence of more than 30 small bacteria per oil immersion field earned 4 points and the absence of small bacteria earned 0 points. The existence of curved rods (Mobiluncus spp) earned an additional 1 or 2 points, depending on the amount of curved rods in each field of vision.

When the points were added together, a total score of

- 0-3 was considered normal.
- 4-6 was classified as intermediate BV positive
- 7-10 was consistent with BV

For our study we will consider the results based in the Nugent criteria.

3.9 Outcome parameters

For our study, the outcome parameters were the adverse pregnancy outcomes that can be associated with BV. We measured the association of spontaneous abortions, still birth and ectopic pregnancy with BV. Another outcome variable 'adverse pregnancy outcome' was developed which included any female who had a past history of either abortions, still birth or ectopic pregnancy. Spontaneous abortion, also known as miscarriage, was defined as the unintentional expulsion of an embryo or fetus before the 24th week of gestation. When a fetus died in utero after the 24^{th} week of gestation during delivery, it was termed "stillborn". Ectopic pregnancy was defined as a condition in which the embryo was attached outside the uterus.

3.10 Variables of Analysis

For the analysis purpose females diagnosed with BV based on Amsel criteria or the Nugent scoring criteria were considered positive for BV. For the Amsel criteria, any female fulfilling three or four Amsel criteria was considered positive for BV and for Nugent Scoring criteria, women with a Nugent score of 5 and more were considered BV positive. A new variable was created, by combining Nugent score and Amsel criteria. It included any female who was positive for Amsel or Nugent criteria. As Nugent criteria is the standard laboratory method for diagnosing BV, we will consider it as the gold standard for our results.

Categorical variables were created in order to analyze the data set using chi square test and run a logistic regression to determine the adverse pregnancy outcomes. The age of females was divided into two categories: females with ages greater than 30 years and ages less than 30 years as is mentioned in the results section. Economic status of women was also divided into high and low. The female belonging to low economic status has a monthly household income of less than 15,000 rupees and women belonging to the high socioeconomic group had a monthly

income between 15,000-20,000 rupees. Age at which women became sexually active was divided into greater than 20 years old and less than 20 years old. Education was divided into two categories, women who were not educated/can read Quran only and the educated women. A new categorical variable "alive children" was created from the continuous variable number of alive children. This variable showed if women had an alive child at the time the study was conducted. The variable "frequency of changing pads in menstrual cycle" was also developed into a categorical variable. Pregnancy status of women was divided into women pregnant or not pregnant at the time the study was conducted. Abortions, still birth and ectopic pregnancy are all the gynecological problems that are caused by various pathological and infectious factors. As posed by the research question, the aim of the study was to determine if there was a relationship between these adverse pregnancy outcomes and BV. Miscarriages and spontaneous abortions were combined to form the variable abortions. A new variable was created by combining abortions, still birth and ectopic pregnancy. The presence of either one or more than one condition was considered as the presence of an adverse pregnancy outcome.

3.11 Statistical Analysis

The study has been approved by the IRB committee at Georgia State University. All the data were analyzed using Statistical Analysis System (SAS). Summary statistics are presented as N and the percentage. Chi-square test (for numbers) was used for bivariate analysis to determine the significance of association between categorical variables. The t-test was used for the univariate analysis of numeric variables. The potential risk factors and covariates, associated with bacterial vaginosis from the literature and results of our study were evaluated by logistic regression analysis. A multivariate logistic regression was run to look at all the adverse pregnancy outcomes of Bacterial Vaginosis individually and as a combination by controlling for

all the socio-demographic and sexual risk factors. The odds ratio for each of the risk factor was also determined. Logistic model was run for each criterion used to define BV (Nugent, Amsel and combined) separately. To determine the association between BV and individual adverse pregnancy outcomes, each adverse pregnancy outcome was run against each of the BV diagnosing criteria.

4.0 RESULTS

The study population comprised of 332 female patients. Age of the patients ranged between 17-42 years with a mean age of 28.0 (SD=0.3) years. About half (48.2%) were sexually active before reaching the age of 20 years, 70.2% had one or more alive child, and 13.9% were pregnant at the at time of the study. About a quarter (27.8%) of the study subjects were illiterate and 42.7% were classified as having low socioeconomic status.

Based on the Nugent scoring system 127 (38.3%) women came out to be BV positive. Based on the Amsel criteria, 113 (34.0%) women were BV positive When we considered the combined basis for the diagnosis of BV, 196 (59.0%) women were positive for BV. The Kappa coefficient for the agreement between Amsel criteria and Nugent criteria came out to be 0.01, showing that there was no agreement between these two criteria for diagnosing BV.

About half (49.4%) of the women had an adverse pregnancy outcome in the past, and abortion was the most prevalent (37.7%). A history still birth was seen in 31(9.3%) females and ectopic pregnancy was also observed in 31 (9.3%) females. On bivariate analyses, educational status (P-value= 0.0026), Economic status (P- value= 0.0010), having alive children (P-value=0.0001) and pregnancy status of women (P-value= 0.0327) showed a statistically significant association with the adverse pregnancy outcomes (Table 1). Diagnosis of BV based on Nugent criteria, Amsel criteria or a combined criterion did not show any significant

association with the adverse pregnancy outcomes.

Table 4.1: Characteristics of the female population presenting with the complain of vaginal discharge with and without the adverse pregnancy outcomes.

Adverse Outcomes	Positive (n=164)	Negative (n=168)	P-value
	N (%)	N (%)	
Age group			0.0812
Less than and equal to 30 years' old	113(68.9)	130(77.38)	
Greater than 30 years	51(31.1)	38(22.62)	
Age at which women becomes sexually active			0.5150
Less than 20 years' old	82(50.0)	78(46.43)	
Greater than 20 year's old	82(50.0)	90 (53.57)	
Educational Status			0.0026
Illiterate or can read Quran	57(35.4)	34(20.48)	
Educated	104(64.6)	132(79.52)	
Economic Status			0.0010
Low	84(51.22)	56(33.33)	
High	80(48.78)	112(66.67)	
Women changes menstrual pad			0.6635
Daily	40(24.39)	40(23.81)	
Once a week	69(42.07)	64(38.10)	
Twice a week	55(33.54)	64(38.10)	
Children alive			0.0001
Yes	131(79.88)	102(60.71)	
No	33(20.12)	66(39.29)	
History of vaginal discharge			0.3645
Yes	19(11.66)	25(15.06)	
No	144(88.34)	141(84.94)	
Women Pregnant			0.0327
Yes	16(9.76)	30(17.86)	
No	148(90.24)	138(82.14)	
Nugent			0.2344
Positive	68(41.46)	59(35.12)	
Negative	96(58.54)	109(64.88)	
Amsel			0.7844
Positive	57(34.76)	56(33.33)	
Negative	107(65.24)	112(66.67)	
BV			0.7921
Positive	98(59.76)	98(58.33)	
Negative	66(40.24)	70(41.67)	

Based on the Nugent criteria, women with and without bacterial vaginosis were comparable in terms of age, age of coitarche, education, economic status, and the frequency of changing menstrual pads during menstrual cycle. Bivariate analysis using chi square test showed

none of the predictors to be significantly associated with bacterial vaginosis except for the history of vaginal discharge (P value=0.0023). None of the adverse pregnancy outcomes as a combined category or as a solitary outcome showed a significant association with BV (Table 2,3).

Table 4.2: Characteristics of the female population presenting with the complain of vaginal discharge diagnosed with BV based on the Nugent score.

Characteristics	BV + (n=127)	BV – (n=205)	P-value
	N (%)	N (%)	
Age group			
Less than and equal to 30 years' old	86 (67.72)	157 (76.59)	0.0762
Greater than 30 years	41 (32.28)	48 (23.41)	
Age at which women becomes sexually active			
Less than 20 years' old	68 (53.54)	92 (44.88)	0.1246
Greater than 20 year's old	59 (46.46)	113 (55.12)	
Educational Status			
Illiterate or can read Quran	41 (32.28)	50 (25.00)	0.1520
Educated	86 (67.72)	150 (75.00)	
Economic Status			
Low	58 (45.67)	82 (40.00)	0.3093
High	69 (54.33)	123 (60.00)	
Women changes menstrual pad	29(22.83)	52 (24.88)	0.8984
Daily	51 (40.16)	82 (40.00)	
Once a week	47 (37.01)	72 (35.12)	
Twice a week			
Children alive			
Yes	94 (74.02)	33 (25.98))	0.2293
No	33(25.98)	66(32.20)	
History of vaginal discharge			
Yes	26(20.63)	18(8.87)	0.0023
No	100(79.37)	185(91.13)	
Women Pregnant			
Yes	12(9.45)	34(16.59)	0.0674
No	115(90.55)	171(83.41)	
Adverse Outcomes	68 (53.54)	96 (46.83)	0.2344
Yes	59 (46.46)	109 (53.17)	
No			

Table 4.3: Comparison of the pregnancy outcomes of the female population presenting with the complain of vaginal discharge when diagnosed with BV based on Nugent score, Amsel criteria or both criteria.

Adverse Pregnancy Outcomes	BV + N (%)	BV – N (%)	P value
Amsel Criteria			
Abortions	43 (38.05)	82(37.44)	0.9134
Ectopic pregnancy	13 (11.5)	18 (8.22)	0.3296
Still birth	12 (10.62)	19 (8.68)	0.5641
Nugent scoring			
Abortions	54(42.52)	71 (34.63)	0.1495
Ectopic pregnancy	11 (8.66)	20 (9.76)	0.7390
Still birth	13 (10.24)	18 (8.78)	0.6577
BV			
Abortions	76 (38.78)	49 (36.03)	0.6166
Ectopic pregnancy	16 (8.16)	15 (11.03)	0.3774
Still birth	21 (10.71)	10 (7.35)	0.3006

Diagnosis of BV based on Amsel criteria showed age (P -value= 0.0071), age of sexual coitrache (P-value= 0.0499) and the frequency with which women changed the menstrual pad in menstrual cycles (P-value= 0.0119) to be statistically significantly associated with BV. Other population characteristics did not show a significant association with BV. Adverse pregnancy outcomes as a combined category or as individual outcomes did not come out be statistically significant (Table 3,4)

Table 4.4: Characteristics of the female population presenting with the complain of vaginal discharge diagnosed with BV based on the Amsel Criteria.

Characteristics	BV + (n=113)	BV – (n=219)	P-value
	N (%)	N (%)	
Age group	93 (82.30)	150 (68.49)	0.0071
Less than and equal to 30 years' old	20 (17.7)	69 (31.51)	
Greater than 30 years			
Age at which women becomes sexually active			
Less than 20 years' old	46 (40.71)	114 (52.05)	0.0499
Greater than 20 year's old	67 (59.29)	105 (47.95)	
Educational Status			
Illiterate or can read Quran	29 (25.66)	62 (28.97)	0.5256
Educated	84(74.34)	152 (71.03)	
Economic Status			
Low	55 (48.67)	85 (38.81)	0.0847
High	58 (51.33)	134 (61.19)	
Women changes menstrual pad			
Daily	37 (32.74)	43 (19.63)	
Once a week	35 (30.97)	98 (44.75)	0.0119
Twice a week	41 (36.28)	78 (35.62)	
Children alive			
Yes	85 (75.22)	148 (67.58)	0.1493
No	28 (24.78)	71 (32.42)	
History of vaginal discharge			
Yes	13 (11.5)	31(14.35)	0.4712
No	100 (88.5)	185 (85.65)	
Women Pregnant			
Yes	18 (15.93)	28(12.79)	0.4320
No	95(84.07)	191(87.21)	
Adverse Outcomes	57 (50.44)	107 (48.86)	0.7844
Yes	56 (49.56)	112 (51.14)	
No			

Population considered positive for BV based on either Nugent score or Amsel criteria, did not show any significant association of the population characteristics with BV except for the history of vaginal discharge (P-value=0.0023) that showed a significant association with BV. None of the adverse pregnancy outcomes as a combined category or as individual outcomes showed a significant association with BV on the bivariate analysis (Table 3,5).

Table 4.5: Characteristics of the female population presenting with the complain of vaginal discharge when diagnosed with BV based on either Nugent Score or Amsel criteria.

Characteristics	BV + (n=196)	BV – (n=136)	
	N (%)	N (%)	P-value
Age group			
Less than and equal to 30 years' old	145 (73.98)	98 (72.06)	0.697
Greater than 30 years	51(26.02)	38 (27.94)	
Age at which women becomes sexually active			
Less than 20 years' old	92 (46.94)	68 (50.0)	0.5830
Greater than 20 year's old	104 (53.06)	68 (50.0)	
Educational Status			
Illiterate or can read Quran	58 (29.59)	33 (25.19)	0.3842
Educated	138 (70.41)	98 (74.81)	
Economic Status			
Low	90 (45.92)	50 (36.76)	0.0967
High	106 (54.08)	86 (63.24)	
Women changes menstrual pad			
Daily	51 (26.02)	29 (21.32)	
Once a week	73 (37.24)	60 (44.12)	0.4091
Twice a week	72 (36.73)	47 (34.56)	
Children alive			
Yes	94 (74.02)	139 (67.8)	0.2293
No	33 (25.98)	66 (32.20)	
Past history of vaginal discharge			
Yes	26(20.63)	18(8.87)	0.0023
No	100(79.37)	185(91.13)	
Women Pregnant			
Yes	12(9.45)	34(15.59)	0.0674
No	115(90.55)	171(83.41)	
Adverse Outcomes			
Yes	98(50.00)	66 (48.53)	0.7921
No	98 (50.00)	70 (51.47)	

Multivariable analyses showed no statistically significant associations between BV and the aggregate adverse pregnancy outcomes. Among the population characteristics, economic status and having an alive child showed significant associations with the adverse pregnancy outcomes (Table 6).

Table 4.6: Adjusted Odds ratio of for Adverse pregnancy outcomes

Characteristics	OR (CI) (Amsel)	P value	OR (CI) (Nugent)	P value	OR (CI) (Combined)	P value
Negative	1		1		1	
Positive	0.96(0.571,1.600)	0.8642	1.12(0.687,1.829)	0.6471	0.87 (0.535,1.405)	0.5615
Age group Less than and equal to 30 years' old	1		1		1	
Greater than 30 years' old	1.39(0.795,2.442)	0.2464	1.39(0.797,2.419)	0.2466	1.39(0.802,2.428)	0.2387
Age at which women becomes sexually active						
Less than 20 years' old	1		1		1	
Greater than 20 year's old	1.08(0.646,1.810)	0.7661	1.09(0.652,1.818)	0.7464	1.08 (0.648,1.799)	0.7669
Educational Status						
Illiterate or can read Quran	1		1		1	
Educated	0.69(0.383, 1.254)	0.2260	0.69(0.383,1.215)	0.2232	0.69(0.382,1.249)	0.2214
Economic Status						
Low	1		1		1	
High	0.45(0.255,0.803)	0.0067	0.46(0.262,0.807)	0.0068	0.45(0.253,0.789)	0.0055
Women changes menstrual pad						
Daily	1		1		1	
Once a week	0.77(0.395,1.1491)	0.9756	0.78(0.405,1.488)	0.9959	0.76 (0.396,1.463)	0.9555
Twice a week	0.60(0.321,1.116)	0.1274	0.60(0.324,1.116)	0.1262	0.60 (0.322,1.108)	0.1282
Children alive						
No	1		1		1	
Yes	2.18(1.281,3.718)	0.0041	0.17(0.077, 0.365)	0.0043	2.21 (1.297,3.756	0.0035
Past history of vaginal discharge						
No	1		1		1	
Yes	0.80(0.398,1.617)	0.5384	0.78(0.379,1.591)	0.4892	0.82(0.406,1.675)	0.5803
Women Pregnant						
No	1		1		1	
Yes	0.55(0.260,1.160)	0.1162	0.58(0.263,1.178)	0.1256	0.54(0.258,1.149)	0.1107
Hosmer Lemshow value		0.2012		0.3526		0.2500

Specific outcome analyses showed no significant association between BV and abortions, still birth or ectopic pregnancy. On the sensitivity analysis using Amsel criteria or the Nugent criteria or both criteria to define BV, the results did not change (Table: 7).

Table 4.7: Adjusted Odds ratio for specific adverse pregnancy outcomes of Bacterial Vaginosis (Amsel criteria, Nugent scoring, Combined).

Pregnancy Outcomes	OR	CI	P value
Abortions			
Amsel	0.857	0.506,1.451	0.5655
Nugent	1.210	0.738,1.986	0.4503
BV	0.890	0.544,1.455	0.6414
Still Birth			
Amsel	1.732	0.721,4.161	0.2197
Nugent	0.988	0.436,2.237	0.9764
BV	1.545	0.668,3.576	0.3093
Ectopic Pregnancy			0.4154
Amsel	1.430	0.604,3.386	0.8732
Nugent	0.934	0.405,2.157	0.4334
BV	0.720	0.316,1.638	

N.B.: - the estimates are adjusted for age of women, age of coitrache, educational status of women, economic status, frequency of changing menstrual pads, number of children alive, pregnancy status of women and history of past vaginal discharge

5.0 DISCUSSION

The present study was conducted on females belonging to the reproductive age group who presented in a public sector hospital with the complain of vaginal discharge. The age of participants (332) ranged from 17-42 years. They were all symptomatic females. Vaginal complaints and vaginal discharge in sexually active females is a global problem and is one of the most common reasons for women to seek gynecological consultation and help. Vaginal complaints and discharge accounts for approximately 10 million visits to the health clinics annually (Anderson 2004).

5.1 Prevalence

The prevalence of BV in the study came out to be 38.25% on the basis of Nugent criteria, which is the gold standard laboratory method of diagnosing BV. A study in the United States has found prevalence of BV among females of age 14-49 years to be 29.2% utilizing the Nugent scoring criteria to diagnose BV. The prevalence of BV is different according to the clinical settings, it ranges from 17-19% in the family planning clinics and 24-47% in the sexually transmitted clinics (Koumans et al. 2007). In India, that has a population similar to this study population in terms of ethnicity and populations characteristics, the prevalence of BV in the married population has been reported to be 20.5%-48.5% (Mohanty and Srujana 2010). In the present study prevalence of BV in pregnant female population came out to be 13.27% which is close to the range of other local studies that have determined the prevalence of BV in pregnant females to be from 17.3% to 64.3% based on the diagnosis of BV on Nugent score. One of the studies had a prevalence in the upper end of the range, i.e. 64.3% which is way higher than the prevalence of BV in the pregnant female population of this study. The differences in the prevalence can be due to the differences in the populations studied, the inclusion of all pregnant females in the study or a mixed population of pregnant and non-pregnant females and the presence of associated diseases like HIV and other STDs (Afolabi et al.2016). Amsel criteria showed the prevalence of BV in this study population to be 34.04%. A study conducted in Iran showed a prevalence of BV to be 37.7% based on the Amsel criteria, which is very close to the results of the present study. These findings are also very close to the studies conducted in Jordan in 2001, Denmark in 2002 and Indonesia in 2001 (Ganjoel 2005).

5.2 Amsel and Nugent scoring

BV causes a change in the vaginal ecosystem and in the present study the change in the vaginal flora related to BV was described by both the Amsel and the Nugent Scoring criteria (Ganjoel 2005). The conventional method of diagnosing BV has been the Amsel criteria, requiring the fulfilment of three of the following four criteria (Amsel 1983): a pH > 4.5, an amine or fishy odor coming from the vaginal discharge on performing the Whiff test, homogeneous consistency of the discharge and presence of clue cells on microscopy (Lata et al. 2010). In the present study all patients were followed by the application with Amsel clinical analysis to separate women suffering from BV from those who had vaginal discharge due to other reasons. In this study population foul odor was observed in 63.25% of the females, thick and homogenous discharge was observed in 66.2% of the females, 25.9% of the females had clue cells in their vaginal discharge and 56.3% of the females had the vaginal pH>4.5. In total, according to the Amsel criteria, 34.04% females were positive for BV.

It is difficult to standardize clinical signs of BV as they depend on the ability and expertise of the clinicians and impossible to be interpreted in a standardized format. BV is often misdiagnosed based on Amsel clinical criteria since the components of clinical diagnosis are subjective and individual to the clinician. In the present study the results of Nugent scoring were more accurate as compared to Amsel clinical criteria as Nugent scoring criteria is a laboratory method of diagnosis. The slides were confirmed by an expert microbiologist, therefore, its inter rater reliability and reproducibility was better than Amsel criteria. Amsel criteria is not the best method to diagnose patients for BV because approximately 50% of the BV patients are asymptomatic (Mohanty and Srujana 2010). Microscopy results, using standard diagnostic method, the Nugent scoring, to detect BV was comparable with other studies in demonstrating the dynamic nature of the microbial population of the vaginal flora (Kimberlin and Andrews).

The Nugent scoring system evaluates the number of Lactobacilli, Gardnerella and Mobiluncus morphotypes on the oil immersion lens under the microscope.

In the present study, for lactobacilli, a score of '0' was given to 12.65% of the females, '1' was given to 13.55 % of the females,' 2' was given to 21.4% of the females, '3' was given to 17.77% of the females and '4' was given to 34.6% of the female population. For GNR, the score '0' was given to 45.18% of the females, score'1' was given to 5.12% of the females, '2' was given to 15.66% of the population, '3' was given to 16.87% of the population and '4' was given to 17.17% of the population. For Mobiluncus, a score '0' was given to 80.36% of the females, '1' was given to 11.78% of the females and '2' was given to 7.58% of the females. In total the female population that came out to be positive for BV when diagnosed on the basis of Nugent Score system with a Nugent score of '5' or more was 38.25%.

The population that was positive for BV based on the combined collective criteria, i.e. positive for Amsel or Nugent criteria came out to be 59.04%.

5.3 Risk Factors

Many risk factors have been associated with BV and with the adverse pregnancy outcomes of BV. These include age of women, age of first sexual intercourse, education, economic status, pregnancy status, number of alive children and the use of menstrual pads.

Based on either criterion to define BV, majority of the females positive for BV were in the lower age bracket (less than 30 years old). Diagnosis of BV based on Amsel criteria, showed age to have a significant association with BV on the bivariate analysis (P-value=0.0071). Literature has also supported the fact that BV is more common in females who are young, less than 30 years of age as compared to other STIs that are more common in older age group

(Klebanoff et al. 2004). However, many studies have found age to not be a significant factor associated with BV. In a study by Holzman et al. (2001), age of the patients came out to be unrelated to the diagnosis of BV as can be seen in the results of bivariate analysis for the present study population when Nugent or combined criteria was used to diagnose BV. A study by Ganjoel (2005), also showed age to have no correlation with BV which supports the results in the current study population.

In this study, the results of multivariate analysis showed greater odds of adverse pregnancy outcomes in BV positive females belonging to the age group of more than 30 years. A study by Morris et al. (2001) has supported the results of the current study. Their study showed that BV was more prevalent in females who were older than 25 years of age. In another study BV has been shown to be more significantly associated with females belonging to the age groups of 35-44 years (Morris et al. 2001).

In this study, diagnosis of BV based on the Amsel criteria, showed age of coitarche to have a significant association with BV (P-value=0.0499). Many studies have shown age of first sexual intercourse to be significantly associated with BV. On the contrary many other studies have shown age of fist sexual encounter to not be associated with BV. A study by Gillet et al. (2011), has mentioned early age at the first intercourse to be a potential risk factor for BV. Risk factor studies of BV in Sweden have also demonstrated young age at first coitrache to be significantly associated with BV. In the present study Nugent scoring or combined criteria of diagnosing BV has not shown age of coitrache to have a significant association with BV. A study trying to determine if BV was a sexually transmitted disease showed no consistent patterns of association between BV and the age of first sexual intercourse (Morris et al. 2001). Studies have

shown that the prevalence of BV rises with the increase in the number of sexual life partners and is more common in those women who have the lower age of first sexual intercourse.

In the current study when BV was diagnosed using Nugent criteria, education and income came out to be statistically significant on the univariate analysis. Studies have shown BV to be more common in women with lower education and income level, hence belonging to a lower socioeconomic strata of the society (Holzman et al. 2001). In this study the results of the multivariable analysis showed that women with BV (diagnosed on any of the three criteria) who were educated had lesser odds of developing adverse pregnancy outcomes as compared to women who were uneducated. The odds ratio for education were significantly associated with BV for all the three criteria of diagnosing BV. Lower educational level has been found to be significantly correlated to BV in a study conducted by Yzeiraj et al. (2013). In the current study, economic status was significantly associated with the adverse pregnancy outcomes in the female population (P-value=0.0010). A study by Hillier et al. (1996), also showed BV to be more common in women belonging to the low income group as it has been shown by many other studies. BV has also been associated with black ethnicity and the women belonging to the lower socioeconomic strata of the society (Morris et al. 2001).

In the present; study, having one or more alive child indicated a previous successful pregnancy and it was found to be significantly associated with the adverse pregnancy outcomes (P-value=0.0001). Adjusted odds ratio for adverse pregnancy outcomes in females diagnosed with BV based on Amsel criteria remained significant for women with one or more alive child (OR= 2.182, 95% CI: 1.281,3.718). The combined criteria of diagnosing BV also showed the increased odds of having adverse pregnancy outcomes in women with one or more alive child (OR= 2.208, 95% CI: 1.297,3.756). However, according to Nugent criteria there were decreased

odds (OR= 0.168, 95% CI: 0.077, 0.365) of having adverse pregnancy outcome in females who have one or more alive child. BV has been significantly associated with having had a prior pregnancy in a study by Koumans et al. (2007). Prevalence of BV has been associated positively with a history of prior pregnancies and increased number of alive children (Holzman et al. 2001). However, for the current study, two out of the three criteria of diagnosing BV supported the findings of previous studies.

In this study, currently pregnant women were significantly associated with adverse pregnancy outcomes (P-value=0.0327). BV is one of the most common genital infections to occur in pregnancy. In previous studies it has been estimated that 12-22% of the pregnant women have BV during pregnancy. It was present in 16% of the study population for a study conducted by Hillier et.al, 1996. (Hillier SL, et al. 1996). This study showed that when Nugent criteria and the combined criteria were used for diagnosing BV, past history of vaginal discharge was significantly associated with BV (P-value=0.0023). Moreover, the frequency with which women changed menstrual pads during menstrual cycles was also significantly associated with BV when diagnosed based on the Amsel criteria (P-value=0.0119).

5.4 Adverse Outcomes

A cause and effect relationship between bacterial vaginosis and adverse pregnancy outcomes can be determined only in prospective studies in which bacterial vaginosis is diagnosed before the onset of pregnancy complications (Kurki et al. 1993). For the present study, adverse outcomes were studied as individual entities: abortions, still birth and ectopic pregnancy and also as a combined entity (presence of either of the mentioned adverse outcome). This study tried to look at the association of the history of past pregnancy outcomes and current diagnosis of BV in women at the time the study took place. The reason for spontaneous abortions is still not

well understood and most of the women are told that a natural selection has taken place and various immunologic or polygenetic factors result in spontaneous abortion. BV has been seen to be 7 times more common in the group of women with a pregnancy loss, and there seems to be no viable explanation to it (Donders et al. 2000). In this study increased odds for abortions (OR=1.210, CI:0.506,4.451) were seen in people with bacterial vaginosis compared to the people without bacterial vaginosis when diagnosed with BV on the basis of Nugent scoring system, the gold standard criteria. In the previous studies, BV has been significantly associated with the occurrence of spontaneous abortions with odds ratio ranging between 6.02-7.31. Abortions has the highest association with BV when compared to any other adverse outcomes. (Leitich et al. 2003).

Another study conducted in a teaching hospital in Leeds has shown increased risks for abortions in women with BV as compared to women without BV. This was the first study to establish a definitive association between BV and spontaneous abortions. It has yet to be established in the future studies if the currently diagnosed BV is developed subsequent to the miscarriages or if it is a reflection of longstanding BV that might have been responsible for miscarriages (Ralph et al.1999). Another study done by Leitich et al. (2007), has also shown BV to be significantly associated with miscarriages. In another meta-analysis to summarize the role of infection in miscarriage, Giakoumelou et al. (2015), mentioned BV to be significantly associated with miscarriages and premature delivery.

In this study increased odds for still birth were observed in females with bacterial vaginosis when diagnosed on the basis of Amsel criteria (OR=1.732, CI: 0.721, 4.161) and combined criteria (OR= 1.545, CI: 0.668,3.576) (Amsel or Nugent), but these odds were not significantly associated with adverse pregnancy outcomes (P-value >0.05). The results of our

study showed increased odds for ectopic pregnancy in women diagnosed with BV based on the Amsel criteria (OR= 1.430, CI: 0.604,3.386), but these odds were also not significantly associated with BV (P-value >0.05). A study by Ganjoel (2005), showed BV to be significantly associated with adverse pregnancy outcomes across all gestational ages. This study also emphasized that BV was more common in women who have had at least one miscarriage. Studies have shown that Nugent defined BV has been significantly associated with increased risks for spontaneous preterm births specially when BV is diagnosed during early pregnancy (Nelson et al. 2016).

Overall, this study revealed a mixed result of BV having an association with adverse pregnancy outcomes in females, based on any of the diagnosing criteria. Economic status was found to be significantly associated with the adverse pregnancy outcomes in females diagnosed with BV. On the multivariable analysis, presence of one or more alive child was significantly associated with the adverse pregnancy outcomes in women diagnosed with BV (P-value=0.0035).

Many studies also support the results of our study. Morris et al. (2001), have reported that BV has not been confirmed to be an independent risk factor for miscarriage, infertility and PID in the absence of STDS. In a study by Nejad and Shafaiq (2008), no significant difference was found in the prevalence rates of BV between pre-term cases with different history of abortions. In a study by Yzeiraj et al. (2013), BV, history of preterm labor in previous pregnancy and low education levels were found to be the independent risk factors for preterm birth but not abortions. Adverse pregnancy outcomes looked at by Yzeiraj et al. (2013), showed BV to not be significantly associated with adverse pregnancy outcomes and specially miscarriages as was observed in a study conducted in Nigeria by Afolabi et al. (2016), where only 4.7% of the BV

positive women in the study population had miscarriages. Adesiji who also conducted a study in South West Nigeria, found no association between adverse pregnancy outcomes and BV. No significant association was found between BV and spontaneous abortions in a study conducted by Afolabi et al. (2016). In a study by Kurki et al. (1993), BV was found to predict the adverse pregnancy outcomes (preterm labor, preterm birth, premature rupture of membranes) poorly and hence no association was developed between BV and adverse pregnancy outcomes. In a study conducted in Kigali University Teaching Hospital in Rawanda, a history of still births was strongly associated with secondary infertility, which was related to the BV infection in these women along with infection from other STIs (Dhont et al.2011). In a study by Hillier et al. (2017) a lack of significant association was observed between BV and the premature rupture of the membranes.

BV itself was not solely related to the history of still births. In a study by Shahgeibi et al. (2009) no statistically significant relationship was observed between BV and abortions. Results from a study conducted by Goffenfg et al. also showed no association between BV and pre term labor. A study in Toronto showed no decrease in the risk of preterm labor after women had been treated for BV. A study by Oakeshott et al, also denied any significant association of BV and spontaneous abortions occurring before the 16th week of gestation. A study by Liversedge also showed no significant association between Bv and abortions (Shahgeibi et al. 2009)

There are a number of potential limitations to this study that could result in the contradictory results for our adverse outcomes. This was a cross sectional study, whereas most of the studies trying to determine the association between BV and adverse pregnancy outcomes are longitudinal and follow up studies. The sample size for this study population was not large enough. It was an observational study, therefore, it made it impossible to exclude unmeasured

confounding. The study was not powered enough. Moreover, the information regarding the adverse pregnancy outcomes was based solely on the subject's survey interview and therefore, a recall bias could have occurred. No hospital record was sought to confirm if the women had undergone an abortion, still birth or ectopic pregnancy. Since all the women who were a part of the study presented with vaginal discharge, there is a possibility that our results could be contaminated by the presence of other sexually transmitted infections or other conditions causing the vaginal discharge and complaints. The criteria of diagnosing BV also affects the results in this study. In this study population, all females presented with the vaginal discharge, therefore, we did not have a control population. The women were not asked around their sexual relationships with other women.

6.0 CONCLUSION

Studies indicate that adverse pregnancy outcomes likes abortions, preterm labor, premature rupture of membranes and low birth weight infant are all the gynecological complications caused by BV. In this study, however, history of the adverse outcomes, as specific outcomes or as a combined outcome showed no significant association with BV. Moreover, Adjusted Odds ratio for specific adverse pregnancy outcomes of Bacterial Vaginosis controlling for age of women, age of coitarche, educational status of women, economic status, frequency of changing menstrual pads, number of children alive, pregnancy status of women and history of past vaginal discharge did show mixed results. In the current study, the odds of abortions were higher in women diagnosed with BV based on Nugent criteria, odds of still birth were higher when diagnosed on the basis of Amsel and combined criteria and the odds of ectopic pregnancy were higher when diagnosed on the basis of Amsel criteria. Our results are not significant (P-

values> 0.05). The study conducted by Afolabi et al. (2016), showed that BV has no association with miscarriage or the admission of neonates to the intensive care units. Another study by Ganjoel 200,5 showed a higher prevalence of BV in patients who has a history of abortions as compared to women who did not have a history of abortions, but this difference was not statistically significant. A number of studies conducted by Goffenfg et al., Oakeshott et al., and Liversedge showed no significant associations between BV and the adverse pregnancy outcomes (abortions and preterm labor)

This study just adds to the literature by supporting that BV is not significantly related to any of the adverse pregnancy outcomes including abortions, ectopic pregnancy and still birth. However, we can suggest that screening of BV is required in fertile women specially with a history of abortions, still birth and ectopic pregnancy to prevent these complications in any new pregnancy. Odds of Adverse pregnancy outcomes are increased due to BV and therefore, BV needs to be screened and treated for.

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