Prevalence and risk factors for bacterial vaginosis in sexually active females in age group 20-45 years and comparison of Amsel's criteria with Nugent's score

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INTRODUCTION

About one fourth of women who attend the Gynaecology OPD complain of vaginal discharge (leucorrhoea) and vaginitis continues to be the most prevalent infection of the female genital tract. Leucorrhoea is a common gynaecological symptom causing annoyance as well as apprehension to the patients.1

The major causes of pathological discharge per vaginum are:

• Trichomonas vaginalis
Candida albicans

Bacterial vaginosis

Bacterial vaginosis is also called non-specific vaginitis, develops when the normally predominant hydrogen peroxide producing lactobacillus species in the vagina are replaced by mixed predominantly anaerobic flora, consisting of Gardnerella vaginalis, Mycoplasma hominis, Mobiluncus species, Bacteroides species, Prevotella species, Peptostreptococcus species, Fusobacterium species and porphyromonas species. Bacterial vaginosis is one of the most common lower genital tract infections among women of childbearing age and yet its diagnosis can be problematic. Bacterial vaginosis has been associated with behavioural, environmental or hormonal factors. Bacterial vaginosis is associated with cervicitis, salphingitis, endometritis, postoperative infections, Urinary tract infections, pelvic inflammatory infections, spontaneous abortions, preterm delivery, abnormal Pap smears and increased susceptibility to HIV. In recent years Bacterial vaginosis has emerged as a global issue of concern due to its association with ascending genital tract infections and with sexually transmitted diseases. So it is important to know the prevalence of Bacterial vaginosis so that proper management can be done. This study is done to establish the prevalence and risk factors of Bacterial vaginosis among sexually active reproductive age females of age group 20-45 yrs attending Gynaecology OPD in SJMCH, Bangalore and to identify the risk factors associated with Bacterial vaginosis.

METHODS

This Prospective observational study was conducted in the gynaecological outpatient clinic of the Department of Obstetrics and Gynaecology at St. John’s Medical College Hospital, Bangalore. All ethical considerations and obligations were duly addressed. Duration of study was over a period of 1 year.

Inclusion criteria

All sexually active women of reproductive age group between 20-45 years who had come to Gynaecology OPD and those who gave consent for the study.

Exclusion criteria

Pregnant women as they form a different cohort.

Diagnosis

Sexually active females of age group 20-45yrs attending gynaecology OPD were selected, and the diagnosis of Bacterial vaginosis was determined based on Amsel’s criteria and Nugent score. Diagnosis by Amsel’s criteria was done in gynaecology outpatient department and diagnosis by Nugent criteria was done in microbiology laboratory. Demographic details of the women were entered.

Amsel method

It is a combined clinical and laboratory method in which Bacterial vaginosis was diagnosed if 3 or more of the following findings were present:

Appearance of vaginal discharge

Per speculum examination was performed, vulva was cleaned with a saline soaked swab, Sim’s speculum was inserted into vagina without any lubricating or any antibacterial agents and type of discharge if any noted appearance of homogenous white, thin non-inflammatory vaginal discharge that adheres to the vaginal wall was diagnostic of Bacterial vaginosis.

Vaginal fluid pH

Normal vaginal pH is 3.8-4.2. PH ≥4.5 was taken as a high vaginal pH and it is seen in Bacterial vaginosis.

Sample was obtained from the lateral vaginal wall or posterior fornix and tested with the help of narrow range pH paper.

Clue cells by wet mount preparation

A swab containing vaginal fluid was obtained and immediately placed in 0.5ml of saline and examined under microscope for clue cells, which are vaginal epithelial cells with borders obscured with adherent small bacteria called “clue cells”.

Figure 1: Clue cells.
Whiff test

Adding a drop of 10% KOH to vaginal fluid and sniffing the mixture. The test was interpreted as positive if a fishy aroma was noted.

Nugent score

Bacterial vaginosis was diagnosed by Nugent score after Gram stained smears was examined under microscope.

Gram stain

- Vaginal swab was taken from the lateral or posterior fornix, smear was made, air dried, heat fixed,
- Air dried, heat fixed smear was stained with methyl violet for one minute and washed under slow running water,
- Next Gram’s iodine solution was poured and allowed to act for half to one minute,
- Smear was decolourised with acetone for 10 seconds and washed under slow running water,
- Counter staining was done with Safrannin for 20-30 seconds, washed under slow running water, dried and seen under microscope under oil immersion.

The Nugent method is a standardized point scoring system (0-10) based on the presence of 3 bacterial morphotypes: large Gram-positive rods (Lactobacillus spp), small Gram-negative or Gram-variable coccobacilli (Gardnerella and anaerobic spp), and curved Gram-variable rods (Mobiluncusspp). Nugent score:

- 0-3 = normal flora
- 4-6 = intermediate flora
- 7-10 = Bacterial vaginosis.

Diagnosing criteria

Bacterial vaginosis was diagnosed if:

- Nugent score of 7-10 or
- Nugent score of 4-6 with 3 or more Amsel’s criteria or
- If ≥3 of the Amsel’s criteria are positive.

Statistical analysis

Descriptive statistics reported as numbers and percentage for the categorical variable and chi-square test or fisher’s exact test was done to test the association between the bacterial vaginosis positive and negative with demographical, clinical and laboratory variables, considering the Nugent criteria as gold standard was compared with the Amsel’s criteria positive and negative, then specificity, sensitivity, PPV and NPV is calculated. Statistical analysis was done by using Statistical software STATA/IC version 12.1 and p<0.05 is considered as statistically significant.
Descriptive statistics: risk factors

Age distribution and bacterial vaginosis: Women with bacterial vaginosis were more in the age group of 20 years to 30 years.

Age of menarche and bacterial vaginosis: Age of menarche was not statistically significant

Parity and bacterial vaginosis: This was statistically significant. The women with Bacterial vaginosis were more in multiparous category about 59.46%.

Socio-economic status and bacterial vaginosis: Statistically not significant, but trend shows that Bacterial vaginosis was found to be more in the lower socio-economic group.

Education status and bacterial vaginosis: Bacterial vaginosis was more common in lower education profile women.

Age of 1st coitus and bacterial vaginosis: Age of first coitus was not statistically significant.

LMP days and bacterial vaginosis: 62.16% of women with Bacterial vaginosis had LMP ≤17 days.

WDPV and bacterial vaginosis: This was statistically not significant. About 51.35% with Bacterial vaginosis had vaginal discharge and 48.65% were asymptomatic.

Prior antibiotic use and bacterial vaginosis: Statistically not significant. 37% women with bacterial vaginosis had taken treatment for vaginal discharge.

Per speculum findings of cervix and bacterial vaginosis: Among women who were positive for bacterial vaginosis, 48.65% had healthy cervix, 45.95% had erosion and 5.4% had cervicitis.

Per speculum finding of vagina and bacterial vaginosis: Significant at 8% level. Women with bacterial vaginosis had healthy vagina in 75.68% and vaginitis in 24.32%.

A) Diagnosis of bacterial vaginosis by Amsel’s criteria

pH of ≥4.5 is one of the criteria for diagnosis of bacterial vaginosis. Among 100 patients who were positive for bacterial vaginosis, 91.89% with bacterial vaginosis had pH ≥4.5 and 8.11% had pH of <4.5. Patients who were bacterial vaginosis negative (17.46%) had pH of ≥4.5.

Table 1: pH.

| Outcome diagnosis | BV. negative | BV. positive | p-value |
|-------------------|--------------|--------------|---------|
| pH <4.5           | 52 (82.54)   | 3 (8.11)     | <0.001  |
| ≥4.5              | 11 (17.46)   | 34 (91.89)   |         |

Table 2: Type of discharge.

| Type of discharge | BV. negative | BV. positive | p-value |
|-------------------|--------------|--------------|---------|
| No discharge      | 32 (50.79)   | 1 (2.70)     |         |
| Homogenous        | 12 (19.05)   | 30 (81.08)   |         |
| Curdy             | 10 (15.87)   | 1 (2.70)     |         |
| Greenish and frothy| 3 (4.76)     | 0 (0.00)     |         |
| Mixed             | 6 (9.52)     | 5 (13.51)    |         |

Among women with bacterial vaginosis, 81% had homogenous discharge, which was one of the diagnostic criteria for bacterial vaginosis.

2.7% had no discharge, 13.51% had mixed discharge, 2.7% had curdy discharge and none had frothy greenish discharge.

Table 3: Wet mount for clue cells.

| Wet mount | BV. negative | BV. positive | p-value |
|-----------|--------------|--------------|---------|
| Negative  | 62 (98.41)   | 7 (18.92)    | <0.001  |
| Positive  | 1 (1.59)     | 30 (81.08)   |         |

Wet mount which are vaginal epithelial cells with borders obscured with adherent small bacteria called “clue cells” was positive in 81.08% of women with bacterial vaginosis and was positive in 1.59% of patients who were negative for bacterial vaginosis. 18.92% had false negative.

Table 4: Whiff test.

| Whiff test | BV. negative | BV. positive | p-value |
|-----------|--------------|--------------|---------|
| Negative  | 59 (93.65)   | 3 (8.11)     | <0.001  |
| Positive  | 4 (6.35)     | 34 (91.89)   |         |

Adding a drop of 10% KOH to vaginal fluid and sniffing the mixture.

The test was interpreted as positive if a fishy aroma was noted. 91% of women with bacterial vaginosis had positive whiff test and 8.11% were false negative.

Table 5: Diagnosis by Amsel’s criteria.

| Amsel’s criteria | BV. negative | BV. positive | p-value |
|------------------|--------------|--------------|---------|
| Negative         | 63 (100.00)  | 1 (2.70)     | <0.001  |
| Positive         | 0 (0.00)     | 36 (97.30)   |         |
Bacterial vaginosis was diagnosed if 3 or more of the following findings were present: ph ≥4.5, homogenous discharge, clue cells and Whiff test: 97.3% of women were diagnosed by Amsel’s criteria and 2.70% were false negative.

B) Diagnosis by Nugent’s criteria

Table 6: Diagnosis of bacterial by Nugent’s criteria.

| Nugent score | BV. negative | BV. positive | p-value |
|--------------|--------------|--------------|---------|
| 0-3          | 51 (80.95)   | 4 (10.81)    |         |
| 4-6          | 12 (19.05)   | 9 (24.32)    | <0.001  |
| 7-10         | 0 (0.00)     | 24 (64.86)   |         |

The Nugent method is a scoring system (0-10) based on the presence of 3 bacterial morphotypes: large Gram-positive rods (Lactobacillus spp), small Gram-negative or Gram-variable coccobacilli (Gardnerella and anaerobic spp), and curved Gram-variable rods (Mobiluncus spp). (Nugent score: 0-3 = normal flora, 4-6 = intermediate flora, 7-10 = Bacterial vaginosis). Among 37 patients who were positive for bacterial vaginosis, 64.86% had a score of 7-10, 24.32% had a score of 4-6 and 10.81% had a score of 0-3.

Table 7: Nugent’s criteria.

| Nugent criteria | BV. negative | BV. positive | p-value |
|-----------------|--------------|--------------|---------|
| Negative        | 63 (100.00)  | 13 (35.14)   | <0.001  |
| Positive        | 0 (0.00)     | 24 (64.86)   |         |

Among women with bacterial positive, 64.86% had score of 7-10, 24.32% had score of 4-6, 10.8% had score of 0-3. 4 people who had Nugent score of 0-3 were considered positive for bacterial vaginosis as they satisfied 3 of 4 Amsel’s criteria.

C) Comparison of Nugent’s criteria and Amsel’s criteria

Table 8: Comparison of Nugent’s criteria and Amsel’s criteria.

| Nugent         | p-value |
|----------------|---------|
| Amsel’s criteria |         |
| Positive        | 32 (71.11) | 4 (7.27) | <0.001  |
| Negative        | 13 (28.89) | 51 (92.73) |         |

Nugent’s criteria was considered as a gold standard for diagnosis of bacterial vaginosis in our study, when Amsel’s criteria was compared with Nugent’s criteria, Amsel’s criteria had a positive predictive value = 88.88%, negative predictive value = 79.67%, sensitivity = 71.11% and specificity = 92.73%.

DISCUSSION

In present study we had 37% prevalence and among them 48.65% were asymptomatic.

Bradshaw et al studied 342 women, 157 (46%) were diagnosed with bacterial vaginosis. It was associated with indicators of high sexual behaviour such as new sexual partner and greater number of male partners in the last year, increased number of lifetime sexual partners, less than 13yrs of education, a past history of pregnancy and smoking (<0.05). The mean age (± standard deviation) was 28.8±7.7 years. 138 had Nugent score 7-10, 125(91%) had Nugent score of 7-10 had 3 or more Amsel criteria, and 13 had less than 3 Amsel criteria but were considered positive for bacterial vaginosis in this study. 35 had Nugent score of 4-6, of whom 19 (54%) had 3 or more Amsel criteria, and 16 had less than 3 Amsel criteria; these were considered negative. Use of oral contraceptive was negatively associated with bacterial vaginosis. There was association between bacterial vaginosis and being in the 1st 17 days of menstrual cycle.

In present study, bacterial vaginosis was more common in the age group of 20 to 30 years. 24 had Nugent score of 7-10, among which 23 (96%) had 3 or more Amsel’s criteria and 1 had less than 3 Amsel’s criteria but was considered positive. 21 had Nugent score of 4-6, among which 9 (43%) had 3 or more Amsel’s score and 12 had less than 3 Amsel’s score; these were considered negative. Use of OCP was not negatively associated with bacterial vaginosis. In present study 62.16% of women with bacterial vaginosis had LMP ≤17 days.

Koumans et al found the prevalence of BV was 29.2% (95% confidence interval 27.2%-31.3%) corresponding to 21 million women with BV; only 15.7% of the women with BV reported vaginal symptoms. Prevalence was 51.4% among non-Hispanic blacks, 31.9% among Mexican Americans, and 23.2% among non-Hispanic whites (P<0.01 for each comparison). Although BV was also associated with poverty (P<0.01), smoking (P<0.05), increasing body mass index (χ²P<0.0001), and having a female sex partner (P<0.005), in the multivariate model, BV only remained positively associated with race/ethnicity, increasing lifetime sex partners (χ²P<0.001), and inversely associated with current use of oral contraceptive pills (P<0.005).

In present study, we found that Bacterial vaginosis was statistically significant association with multiparous women. The trend was more towards low socio-economic
Gilbert Donders in his review article found that in Caucasian women the prevalence was 5% to 15%, in African and American blacks 45% to 55%. In Asian women the prevalence was less well studied, but in general was around 20% to 30%. About half of women with BV had symptoms. In present study we had a prevalence of 37% and among them 48.65% were asymptomatic.

Klebanoff et al noted among 2,888 women with bacterial vaginosis, 75% of women with and 82% of women without bacterial vaginosis never noted any vaginal odour in the past 6 months (P < 0.001).

The corresponding values were 63% and 65% for never noting vaginal “wetness” (P = 0.02); 58% and 57% for vaginal discharge (P = 0.65); 91% and 86% for irritation (P = 0.004); 88% and 85% for itching (P = 0.64); and 96% and 94% for dysuria (P = 0.002), respectively. Cumulatively, 58% of women with bacterial vaginosis noted odour, discharge, and/or wetness in the past 6 months compared with 57% of women without bacterial vaginosis (P = 0.70).

In present study among 37 women bacterial vaginosis, 46% with and 65% without bacterial vaginosis never noted any vaginal discharge in the past 6 months. Whereas 54% with and 35% without bacterial vaginosis had vaginal discharge in past 6 months.

Chaijareenont K et al, Considering Amsel's criteria as the gold standard, Nugent's score showed a sensitivity of 65.6% (95% CI 46.8%, 80.8%), specificity of 97.3% (95% CI 93.5%, 99.0%), positive predictive value (PPV) of 80.8% (95% CI 60.0%, 92.7%), negative predictive value (NPV) of 94.2% (95% CI 89.7%, 96.9%) and accuracy of 92.6% (95% CI 88.1%, 95.6%). Both vaginal pH and whiff test demonstrated 100% sensitivity.

However, vaginal pH showed lower specificity than the whiff test (58.9% and 97.3% respectively) and concluded that, Nugent’s score might not be suitable to use as a screening test for diagnosis of BV due to its low sensitivity. The whiff test was the best clinical criteria of Amsel’s criterion in the diagnosis of BV due to its high sensitivity and specificity.

In present study we considered Nugent’s criteria as gold standard and found that Amsel’s criteria had Positive predictive value = 88.88%, Negative predictive value = 79.67%, Sensitivity = 71.11% and Specificity = 92.73%

Smart S et al studied social and sexual risk factors for bacterial vaginosis. They studied 890 women with BV and 890 controls. 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.

Sujatha R et al found among the 300 patients with symptomatic vaginal discharge, 123 (41%) were diagnosed having bacterial vaginosis by Amsel criteria. Out of these 120 (97.5%) patients had homogenous milk like discharge, 112 (91%) had a positive whiff test, 121 (98.3%) had clue cells and 111 [90%] had P>4.5. and 126 [42%] were diagnosed by gram stain.

Eschenback et al compared the diagnosis of bacterial vaginosis on the basis of Amsel’s criteria or presence of “clue” cells alone with Gram stain criteria and isolation of G. vaginalis on culture. They reported that if bacterial vaginosis defined by composite clinical criteria the independent diagnosis of bacterial vaginosis by Gram stain alone had a sensitive of 97% and a specificity of 79% and positive predictive value of 69%.

B Adane in his article found overall prevalence rate of bacterial vaginosis by Gram-stain Nugent scoring criteria was 48.6%, the proportion of bacterial vaginosis was the highest in age groups above 45 years.

In present study, we found that bacterial vaginosis was statistically significantly associated with multipara. OCP and condoms were not found to be protective and also the prevalence of using OCP and condoms was less in our subjects. None of our subjects were smokers and none had multiple partners.

Mohammadzadeh F et al studied the sensitivity, specificity, positive predictive value, negative predictive value, and accuracy of Amsel’s criteria the results were 91%, 91%, 86%, 94%, and 91%, respectively. Moreover, the presence of clue cells in vaginal wet mount and whiff tests had the greatest sensitivity (97.6%) and specificity (85.7%), respectively, vaginal discharge had a sensitivity of 86.7% and a specificity of 56.5% in the diagnosis of BV, vaginal pH had the lowest specificity (46.6%). Various factors such as simultaneous infections of vagina and cervical mucus might had influenced the criterion. The study showed Amsel criteria to have high specificity (91%) and sensitivity (91%) in BV diagnosis. And Amsel criteria could be as good as Nugent scoring system at diagnosing this infection.

In present study authors considered Nugent’s criteria as gold standard and found that Amsel’s criteria had positive predictive value = 88.88%, negative predictive value = 79.67%, sensitivity = 71.11% and specificity = 92.73%.

CONCLUSION

Bacterial vaginosis is associated with cervicitis, salpingitis, endometritis, postoperative infections, Urinary tract infections, pelvic inflammatory diseases, spontaneous abortions, preterm delivery, abnormal Pap smears and increased susceptibility to HIV.
Prevalence of BV among sexually active females in age group of 20-45 years attending outpatient department of OBG, St. John’s medical college hospital was 37%.

Risk factor associated with bacterial vaginosis which was statistically significant was multiparity. Bacterial vaginosis also showed trend towards low socioeconomic and low education status and LMP ≤17 days. It was more common in age group of 20-30 years. Women using IUCD, OCP, Condom were less in present study

Prevalence of Bacterial vaginosis was 37%, which is a significant proportion and therefore screening for bacterial vaginosis may be useful to detect and treat the cases. Amsel’s criteria can be used to clinically diagnose Bacterial vaginosis especially in the out-patient department as it has good specificity. But since the sample size in present study was only 100, larger trials are needed before any recommendation can be made.

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