

The Microbiological Profile of Vaginosis among Women of the Reproductive Age Group, Who Attended a Tertiary Care Hospital

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ABSTRACT

Background: An abnormal vaginal discharge is a common complaint in women and it can be due to vaginal infections like bacterial vaginosis, candidiasis and trichomoniasis .

Aim: A descriptive cross sectional study was done to find out the common pathogens which caused vaginosis in patients of the reproductive age group, who attended the Obstetrics and Gynaecology Department at the Saveetha Medical College Hospital, Thandalam, Kanchipuram District, from 1st April to 30th September, 2010.

Materials and Methods: All the 140 vaginal discharge samples were subjected to gram staining to view the morphological

nature of the bacteria which caused bacterial vaginosis and gram positive, budding yeast cells, wet film for the motility of Trichomonas and Chlamydial antigen detection by ELISA.

Results: The overall positivity was 44%, which included bacterial vaginosis (36.4%). The positivity of candidiasis was 4.2%, that of trichomoniasis was 2.1% and that of chlamydia was 1.4%.

Conclusion: Bacterial vaginosis was found to be the commonest cause of the abnormal vaginal discharge in women of the reproductive age group.

Key Words: Bacterial vaginosis, Nugent's criteria, Vaginal candidiasis, Trichomoniasis, Chlamydia

KEY MESSAGE

- The predominant cause of the vaginosis was bacterial vaginosis. Among the 62 positive vaginosis samples, 51 were due to bacterial vaginosis. The positivity of candidiasis was 4.2%, that of trichomoniasis was 2.1% and that of chlamydia was 1.4%. Candidiasis was the other important cause of vaginosis in the abnormal vaginal discharge patients. Candidiasis was seen more in patients with Diabetic mellitus.

INTRODUCTION

Vaginal infections such as bacterial vaginosis, candidiasis as the common yeast infection, and trichomoniasis, are the common causes of abnormal vaginal discharge [1]. Bacterial vaginosis (BV) is one of the important causes of vaginal infections among women of the child bearing group, because of its potential to cause upper genital tract infection [2]. *Chlamydia trachomatis* today, has become the most common bacterial sexually transmitted infection (STI), all over the world. Most of the times, it is asymptomatic in the female reproductive tract; but if left untreated, it leads to dreaded consequences like pelvic inflammatory disease (PID), infertility, ectopic pregnancy and chronic pelvic pain. It can cause endometritis, inflammation of the tubes, and at times, also peritonitis with the Fitz Hugh Curtis syndrome [3].

Hence, knowledge on the microbiological profile of vaginosis becomes necessary to diagnose and treat abnormal vaginal discharges. A descriptive cross sectional study was done to study the common pathogens which caused vaginosis in patients of the reproductive age group, who attended the Obstetrics and Gynaecology Department at the Saveetha Medical College Hospital, Thandalam, Kanchipuram District, Tamilnadu.

AIM

To identify the common causes of abnormal vaginal discharge, like bacterial vaginosis, vaginal candidiasis, trichomoniasis and chlamydial infections among women of the reproductive age group in a tertiary care hospital.

MATERIALS AND METHODS

The present study was carried out at the Saveetha Medical College, which is a tertiary care hospital in Thandalam, Kanchipuram district. The study period was from 1st April to 30th September, 2010. Vaginal swabs were collected from 140 patients with abnormal vaginal discharge, who attended the Saveetha Medical College and Hospital.

Inclusion Criteria

All the female patients in the reproductive age group (17 to 50 years), with the complaint of excessive vaginal discharge were included in this study.

Exclusion Criteria

1. Patients with bleeding per vagina
2. Patients of age group below 17 years and above 50 years

- Patients with genital tract malignancies (cervical and endometrial carcinomas)

The specimens were collected by using sterile cotton swabs and they were transported to the lab for further processing. The following tests were done in the laboratory according to standard procedures [4,5].

- Smears from swabs
- pH testing
- Gram staining
- Wet mount
- KOH mount
- Whiff test
- Chlamydial antigen detection by using ELISA

For bacterial vaginosis, the results were interpreted by using Nugent's criteria [2], as shown in [Tables/Fig-1 and 2].

RESULTS

Among the 140 cases which were studied, 20 (14%) were inpatients and 120 (86%) were outpatients. The samples were collected from patients of the age group of 17- 50 years. The age wise distribution of the patients is shown in [Table/Fig-3]. The maximum number of patients (26.40%) were from the age group of 36-40 years.

Out of the 140 patients, 80 patients had excessive vaginal discharge and lower abdominal pain. Twenty patients had excessive vaginal discharge and lower abdominal pain along with itching. Itching was the only presenting symptom in 10 patients. Twenty patients complained of a foul smelling discharge along with the itching. Five patients had infertility and 5 patients had menstrual problems like irregular menstruation and excessive bleeding. The presenting symptoms with the number of patients are depicted in [Table/Fig- 4].

The colour and nature of the vaginal discharge with the number of patients are shown in [Table/Fig-5].

The pH of all the samples was tested. The pH distribution is shown in [Table/Fig-6]. Out of the 140 patients, 81 had normal pH (4 and <4). An increase in pH (4.5 and > 4.5) was found in 59 patients.

All the 140 samples were subjected to gram staining, KOH mount, wet film and antigen detection for Chlamydia. Gram staining was done to view the morphological nature of the bacteria which caused vaginosis. The presence of gram variable rods and gram negative rods indicated bacterial vaginosis [Table/Fig-7]. Candida were seen as gram positive budding yeast cells [Table/Fig-8]. KOH mount was also done for observing Candida and wet film was done to study the motility of Trichomonas. Antigen detection by ELISA was done for Chlamydia. The gram stained smear of bacterial vaginosis was scored by using the Nugent's scoring system. The 51 positive cases of bacterial vaginosis were scored as shown in [Table/Fig-9]. The positivity of bacterial vaginosis was 36.4% according to Nugent's criteria.

Among the 140 cases, 62 cases were proven to have vaginosis. The percentage of the positivity was 44%. This positivity included bacterial vaginosis, candidiasis, trichomoniasis and chlamydiasis as depicted in [Table/Fig-10]. The positivity of candidiasis was 4.2%, that of trichomoniasis was 2.1% and that of chlamydiasis was 1.4%.

Among the 51 positive cases of bacterial vaginosis, the bacterium which caused vaginosis in most of the cases was Gardnerella

Bacterial Morpho type	None (Score)	1+	2+	3+	4+
Lactobacilli (large GPB)	4	3	2	1	0
Small GN/ G variable rod	0	1	2	3	4
Curved GNB (Mobiluncus)	0	1	2	3	4

[Table/Fig-1]: Scoring system of Gram stained smears (Nugent et al) [2]

GPB- Gram positive Bacilli.
GNB- Gram negative Bacilli.
GN- Gram negativeve.

Bacteria	Score	Report
<1 / oil immersion field 1+	0- 3	Normal
1- 5 / oil immersion field 2+	4- 6	Intermediate
6- 30 / oil immersion field 3+		Test to be repeated later
>30 / oil immersion field 4+	7- 10	Bacterial vaginosis

[Table/Fig-2]: Scoring system of Gram stained smears

Age in years	Number	Percentage
20	1	0.9
21-25	19	13.5
26-30	29	20.7
31-35	22	15.7
36-40	37	26.4
41-45	18	12.8
46-50	14	10

[Table/Fig-3]: showing the age distribution

Symptom	No of patients
Excessive vaginal discharge & lower abdominal pain	80
Excessive vaginal discharge, Lower abdominal pain & itching	20
Itching	10
Foul smelling discharge	20
Infertility	5
Menstrual problem	5

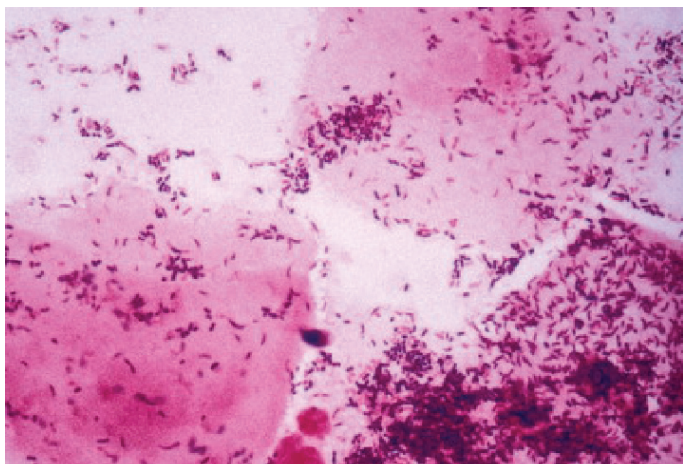
[Table/Fig-4]: Showing presenting features

Color & nature of discharge	Number	Percentage
White & thick	79	56.4%
White & mucopurulent	42	30%
Yellow & thick	15	10.7%
Yellow & frothy	4	2.8%

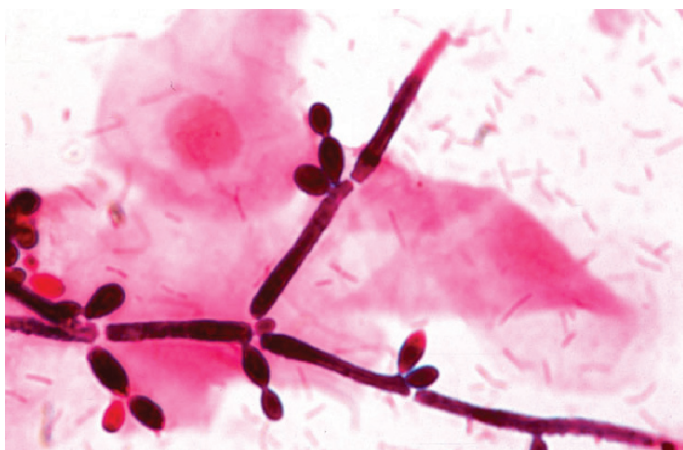
[Table/Fig-5]: Showing nature of discharge

pH	Number
3.5	33
4	48
4.5	6
4.6	13
4.7	16
4.8	16
4.9	2
5	6

[Table/Fig-6]: showing pH



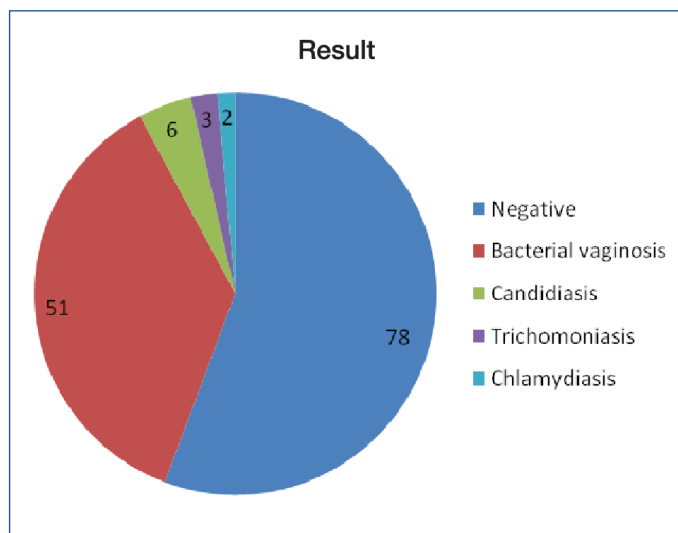
[Table/Fig-7]: Showing Gram variable rods suggestive of Gardnerella vaginalis



[Table/Fig-8]: showing budding yeast cells suggestive of Candidiasis

Nugent's score	Number of cases
4	3
5	2
6	4
7	2
8	28
9	1
10	1
11	5
12	5

[Table/Fig-9]: Showing score by Nugent's criteria



[Table/Fig-10]: Showing the results

(86.2%), which was identified by its morphological features. Mobiluncus (curved gram negative bacilli) was always found in association with Gardnerella in 11.6% cases.

The Whiff's test is found to be positive in all the 51 cases that were positive for bacterial vaginosis. The Whiff's test was found to be negative in the vaginal discharge that was positive for vaginosis due to Candida, Trichomonas and Chlamydia.

Bacterial vaginosis was found to be associated with patients who had a white and mucopurulent discharge, a yellow discharge with Candidiasis and a yellow and frothy discharge with Trichomoniasis. The increase in pH was due to the growth of organisms other than lactobacilli. This led to vaginosis. Clue cells were observed in all the positive cases of bacterial vaginosis. The clue cells were embedded with the gram variable bacterium (Gardnerella).

Candidiasis was the other important cause of vaginosis, where the discharge was yellow in colour. Among the 62 positive vaginosis patients, the number of positivity was 6 (4.2%). It was detected by gram staining and the KOH mount. Candidiasis was seen more in patients with Diabetic mellitus.

Trichomoniasis was found in 3 patients who had a yellowish frothy discharge. It was detected by doing a wet mount preparation by using normal saline. Its positivity was confirmed by its characteristic morphology and its motility under the microscope.

Chlamydia was detected by the rapid antigen detection method. Among 140 patients, 2 were diagnosed to be chlamydial antigen positive. Both these patients had infertility.

DISCUSSION

In the present study, during a period of 6 months (April to September 2010), 140 samples of abnormal vaginal discharge were analyzed. The Nugent's scoring system was employed for diagnosing bacterial vaginosis [2]. The Nugent's scoring system appears to be a reliable and convenient method for the laboratory evaluation of the cases of bacterial vaginosis, as in similar studies which were done by R. Mathew et al, Chennai and Mohanty S, et al, New Delhi [2,6].

Among the 140 cases which were studied, 20(14%) were inpatients and 120 (86%) were outpatients. In our study, out of the 140 samples, the maximum (37) were from the age group of 36-40 years. The most prevalent cause of vaginosis was bacterial vaginosis, which was 36.4% according to Nugent's criteria. The positivity of candidiasis was 4.2%, that of trichomoniasis was 2.1% and that of chlamydisis was 1.4%. Most of the patients complained of excessive vaginal discharge (57%), lower abdominal pain (54.2%), infertility (3.5%) and menstrual problems (3.5%). Most of the patients had combined complaints also.

A similar study which was done by R.Mathew et al, Chennai on the prevalence of bacterial vaginosis in antenatal women, included 150 symptomatic women and 50 asymptomatic women who were in the second trimester of pregnancy and between 20-30 years of age. The smears were examined and evaluated by the scoring system of Nugent et al. In their study, the positivity of the symptomatic women was 38.5% for bacterial vaginosis and the positivity in asymptomatic women was 8%. The morphological predominance of the organisms were Gardnerella (26), followed by

Mobiluncus (20) and clue cells (19) [2]. The percentage of positivity for bacterial vaginosis was 36.4% in our study, which was in accordance with this study.

Thulkar J, et al, New Delhi, studied the utility of the pH test and the Whiff's test in a syndromic approach of abnormal vaginal discharge, whose study included 564 women with abnormal vaginal discharge. Vaginitis was diagnosed in 301 (53.37%) women. Bacterial vaginosis (BV) was the commonest type of vaginitis (39.01%). A pH of $>$ or $=$ 4.5 and a positive Whiff's test had a sensitivity of 94.09 per cent and a specificity of 87.5 per cent in diagnosing bacterial vaginosis. Similarly, a pH of $<$ 4.5 and a positive or negative Whiff's test had a sensitivity of 83.72 per cent in diagnosing candidiasis. They concluded that the pH test and the Whiff's test can improve the diagnostic value for vaginosis [7]. In the present study, the pH test and the Whiff's test were very useful for the diagnosis of bacterial vaginosis. The Whiff's test, if it was poorly positive, the microscopic examination revealed an intermediate stage of bacterial vaginosis. If it was strongly positive, the microscopic finding confirmed bacterial vaginosis. A negative Whiff's test showed a negative microscopic finding. The pH was $>$ than 4.4 in the bacterial vaginosis samples and it was $<$ 4.4 in the normal samples.

Mania-Pramanik J et al studied the use of vaginal pH in the diagnosis of infections and its association with reproductive manifestations. The *C. trachomatis* infection or different reproductive manifestations do not lead to a change in the vaginal pH, but a high vaginal pH was correlated with bacterial vaginosis and it should be used as a simple tool for its diagnosis [8]. A high vaginal pH (more than 4.5) was seen in all the cases of bacterial vaginosis in our study, which was correlated well with the findings of previous studies.

Bacterial vaginosis was found to be the most prevalent (36.4%) form of vaginosis which affected women of the reproductive age group in our study. A related study on the diagnosis and prevalence of vaginosis, which was done by SP Saharan et al, had reported the positivity of bacterial vaginosis as 37.5% [9]. Bacterial vaginosis is generally considered to be a condition of premenopausal women. In our study, the maximum number of patients who were affected were in the age group of 36-40 years. Among the individual criteria which were used to diagnose bacterial vaginosis, a raised pH was recognized as the most sensitive but the least specific criterion. The amine test is both highly sensitive and specific. False positive amine tests occur rarely. The gram stain was strikingly positive in patients with bacterial vaginosis. It was 100% specific and sensitive in comparison with the compound criteria for the diagnosis of bacterial vaginosis. Gram staining is an easy method of diagnosing bacterial vaginosis [10].

Though bacterial vaginosis was found to be the most common cause (36.4%) of vaginosis, which affected women of the reproductive age group in our study, there are a few studies where a higher prevalence of bacterial vaginosis was reported. Bhalla P, et al, New Delhi, studied on the prevalence of bacterial vaginosis among women in Delhi. Vaginal specimens for the gram-stain evaluation of the vaginal flora for the diagnosis of bacterial vaginosis and the culture of *Trichomonas vaginalis* and *Candida* spp, blood samples for the serology of HIV and syphilis and urine for the detection of *Neisseria gonorrhoeae* and *Chlamydia trachomatis* were collected from women (15-49 yrs) from the rural and urban areas. Information on the demographic characteristics, the risk factors and the clinical symptoms was obtained. Bacterial vaginosis was diagnosed in 70 (32.8%) subjects. A high percentage of women, though they were

asymptomatic (31.2%), were found to have bacterial vaginosis. The highest prevalence was seen in urban slums (38.6%), followed by rural (28.8%) and urban middle class communities (25.4%). All the women with vaginal trichomoniasis were found to have bacterial vaginosis, while 50 per cent of the subjects who had syphilis also had bacterial vaginosis. In our study, 90% of the positive BV cases were from rural areas and 10% were from urban areas [11].

In the present study, bacterial vaginosis was found to be mainly due to anaerobic bacteria which morphologically resembled *Gardnerella vaginalis* and *Mobiluncus*. Similar results were seen in a study by Sumati AH et al, where high vaginal swabs were taken from 174 female patients who complained of abnormal vaginal discharge. BV was diagnosed by clinical composite criteria and by gram staining. BV was diagnosed in 68.39% of the cases by using clinical composite criteria and in 58.4% of the cases by gram staining. They concluded that anaerobic bacteria are important pathogens in the causation of bacterial vaginosis, along with other aerobic organisms. The percentages of bacteroides and peptostreptococci were significantly raised in BV [12].

In another study by Uma S et al on bacterial vaginosis in female sex workers in Chennai, they investigated both the prevalence of BV and its association with STDs among 582 female sex workers. The vaginal swabs which were collected were gram stained and analyzed for BV by using Nugent's scoring criteria. Of the women who were studied, 45% were positive, 39.5% were negative and 16% were intermediate for BV [13]. In our study, positive bacterial vaginosis was 42 and the intermediate stage of BV was 9. In these studies, co-morbid conditions were associated with vaginosis, which could be a possible reason for the higher prevalence. The co-morbid conditions included recurrent vaginitis, various contraceptive methods, demographic characteristics, sex workers, etc [10,13].

The percentage of Trichomoniasis was 2.1% in this study, which was similar to the results of some other studies. Sood S et al, New Delhi, who studied on the In Pouch TV culture for the detection of *Trichomonas vaginalis*, revealed that among 601 women, 22 (7%) were found to be positive for *Trichomonas vaginalis* by wet mount detection [14].

In a study by Rao VG et al on sexually transmitted infections in the tribal populations of central India, trichomoniasis, gonorrhoea, bacterial vaginosis and syphilis sero-reactivity were diagnosed by the standard microbiological techniques. Chlamydia infection was detected by using the polymerase chain reaction (PCR). A definite laboratory diagnosis of STIs could be established in 36.5% of the individuals. The most common STI in females was trichomoniasis. The highest proportion of individuals with STIs (39.2%) was in the age group of 30-39 years. When compared to this study, the prevalence of *Trichomonas vaginalis* in our study was found to be low (5%), since it was from the general population. The wet mount detection was not as sensitive as the PCR technique [15].

Vidhani S et al, studied on the seroprevalence of the *Chlamydia trachomatis* infection amongst patients with pelvic inflammatory diseases (PID) and infertility. The study evaluated the seroprevalence of the chlamydial infection in patients with pelvic inflammatory disease (ND) and infertility and in the control population of healthy normal females. The seroprevalence was found to be 82.7% in the patients and 32% in the controls by Enzyme Linked Immuno Sorbent Assay (ELISA) for any one or more classes of antichlamydial antibodies (IgG/IgM/ IgA). This study demonstrates the importance

of serology in monitoring the trends of chlamydial infections in women with PID and infertility. In our study, the rapid detection of the Chlamydial Antigen revealed a 4% positivity which was much lower than that in the study by Vidhani et al. The probable reason was that the population which was studied had PID and infertility in the latter study [16].

CONCLUSION

Bacterial vaginosis was found to be the commonest cause of abnormal vaginal discharge in women of the reproductive age group in our study, followed by Candidiasis.

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