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Research Article

A Study of Bacterial Vaginosis with special reference to Gardnerella vaginalis

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Abstract: In Bacterial Vaginosis, the normal lactobacilli dominated flora is replaced by a mixed predominantly anaerobic flora. There is an increase in the obligatory facultative anaerobes like Gardnerella vaginalis, Bacteroides, Peptostreptococcus, Mobiluncus and Mycoplasma hominis. Of all the microorganisms stated, Gardnerella vaginalis predominates. A total of 127 samples were collected from the patients attending Gynaecology OPD complaining of vaginal discharge, Vulvar irritation that is in the age group of 20-50 years. Two High Vaginal swabs were collected from each patient from posterior fornix. One swab was used for Direct Gram staining and other swab inoculated on Macconkey Agar, Columbia blood Agar and Sabourads dextrose Agar and incubated at 37^0c. Gardnerella vaginalis was identified by biochemical tests. Antibiotic sensitivity testing was done. Gardnerella vaginalis most common pathogenic organism isolated about 28.3%. Gardnerella vaginalis was most common in the age group of 26-30 years (26.7%), followed by 16-20 years (24.4%) and 21-25 years (21.2%). the antibiotic susceptibility of Gardnerella vaginalis revealed that the organism is most sensitive to Metronidazole (94.4%), followed by clindamycin (86.1%), Ampicillin (69.4%), gentamicin (58.3%), ciprofloxacin (47.2%) and Co-trimaxozle (33.3%). Women are less likely to seek treatment for the morbidity and thus are more likely to acquire other serious STI's. In view of this it is suggested that women attending antenatal and gynecology clinics or family planning clinic should be screened and treated for BV cases to reduce risk of other STI.
Keywords: Bacterial vaginosis, Gardnerella vaginalis, vaginal swab

INTRODUCTION:

Bacterial Vaginosis (BV) is a condition of alteration in the normal vaginal ecosystem caused by, a considerable decrease in number of lactobacilli and a 100-fold increase in the growth of both aerobic and anaerobic bacteria flora[1].

It is the most prevalent vaginitis and is responsible for approximately one third of all cases of vulvovaginitis in women of reproductive age group. The hall mark symptoms are excessive discharge and odour. The diagnosis of this condition is likely when a patient complains of a malodorous, non irritating discharge, and on examination reveals homogeneous grey white secretions, but more than one half of patients with demonstrable signs have no symptoms[2].

Bacterial vaginosis is not a notifiable disease hence exact prevalence data is lacking. The prevalence varies from study to study. It is more common in IUCD users, women with increased frequency of coitus and in women with increased number of sexual partners [3].

Many factors have been related to changes in the vaginal flora including menstruation, concomitant infection, sexual activity, smoking, douching, number of sexual partners, and contraceptive methods[4] Sexual transmission of bacterial vaginosis is unclear.

In Bacterial Vaginosis, the normal lactobacilli dominated flora is replaced by a mixed predominantly anaerobic flora. There is an increase in the obligatory facultative anaerobes like Gardnerella vaginalis, Bacteroides, Peptostreptococcus, Mobiluncus and Mycoplasma hominis. There is a decrease in the vaginal lactic acid content associated with an increase in vaginal pH>4.5. The concentration of bacteria increases 100 to 1000 fold. Anaerobic bacteria can be found in less than 1% of the flora of the normal women. Of all the microorganisms stated, Gardenerella vaginalis predominates [4].

Gardnerella vaginalis are small, pleomorphic, gram-negative to gram-variable bacilli [3]. Pheifer *et al.;* [5] noted that a fishy odor was emitted when

vaginal fluid from a woman with BV was exposed to 10% KOH. They also found the Gardnerella vaginalis in the urethra of male partners of patients with G.Vaginalis. They also demonstrated the efficacy of the therapy of NSV [5].

Until recently bacterial vaginosis was regarded as a harmless abnormality and patients were treated only if symptomatic. Recently a variety of complications associated with bacterial vaginosis has been reported in pregnant and non pregnant women. Several bacteria can cause the infection. Those most often mentioned are the various staphylococci, streptococci, coliform bacilli, micrococci and diphtheroids. 40% of G. vaginalis was isolated in IUCD users.

A recently published hypothesis suggests that bacterial vaginosis can cause neoplasia of the cervix [6]. There is a strong development of vaginal cuff infections following hysterectomy and pelvic inflammatory disease has been postulated. One study shows an association between premature labor with intact membranes and amniotic fluid infected with bacteria [7]. In the patients with gynecologic disease, bacterial vaginosis [8] is associated with laparoscopically proved pelvic inflammatory disease, urinary tract infections, endometritis, post partum endometritis and chorioamnionitis [9].

The secret of successful management of vaginal discharges or infections is in the diagnostic approach. If a proper diagnosis is made, treatment follows easily. Although the crux of the diagnosis of vaginal infections rests with the microscopic examination, clinical evaluation plays a vital role.

The aim of this study was to assess the microbial flora in women complaining of vaginal discharge with special reference to nonspecific vaginitis caused by Gardnerella vaginalis along with its Antibiotic susceptibility pattern.

MATERIALS AND METHODS:

A total of 127 samples were collected from the patients attending Gynaecology OPD complaining of vaginal discharge, Vulvar irritation who are in the age group of 20-50 years and not in Menstruating period. These samples were processed in Microbiology department at Narayana Medical College, Nellore. This is a prospective study done for one year in 2012. Patient's informed consent has taken and the ethical committee has approved.

Before proceeding for Sample collection patients detailed history about present complaints along with significant past history like usage of any contraception, sexual history, Medical illness has taken. General examination of patients and Gynaecological examination has done using Sim's speculum under aseptic precautions.

Two High Vaginal swabs were collected from each patient from posterior fornix. Type of Vaginal discharge has noted. The pH was measured using indication papers (Ranbaxy lab) ranging from 1 – 14. Amine test has done using 10% Potassium hydroxide (KOH).

One swab was used for Direct Gram staining and other swab inoculated on Macconkey Agar, Columbia blood Agar and Sabourads dextrose Agar and incubated at 370c.

Identification of Gardnerella vaginalis: Clue cells has noted in Direct Gram staining preparation. Culture plates were examined for the presence of smooth, grayish, white, shiny, opaque colony about 0.5-1mm in size with diffuse β-hemolytic colony on Columbia blood agar, Non lactose fermenting colonies on Mac conkey Agar,. Gram stain was performed and observed for gram negative to gram variable coccobacilli. Catalase & Oxidase tests were performed and found negative. The organism was presumptively identified as Gardnerella vaginalis [10].

Antibiotic susceptibility testing was done for Gardnerella vaginalis on human blood agar by Kirby-Bauer disc diffusion method using Ciprofloxacin-10µg, Ampicillin-25µg, Clindamycin-30µg, Cotrimoxazole-25µg, Gentamicin-10µg, and Metronidazole-50µg.

RESULTS:

Out of 127 samples most of the cases were noticed in Low socioeconomic status about 66.9%. Bacterial vaginosis was correlated with Diabetes mellitus it has shown that 12.5% of cases have DM. various organisms have isolated from 127 samples and percentage of those is depicted in Table No.1. Gardnerella vaginalis most common pathogenic organism isolated about 28.3%.

Gardnerella vaginalis was most common in the age group of 26-30 years (26.7%), followed by 16-20 years (24.4%) and 21-25 years (21.2%). All the 36 isolates of G.vaginalis were shown 100% negative to catalase & oxidase tests. All the isolates fermented glucose, maltose and starch by producing acid. Gardnerella vaginalis detection was assessed by correlating with Spiegel's criteria (Table No.2).

Nugents Scoring was assessed by Direct Gram staining for all suspected Bacterial vaginosis cases (Table No.3).

Table-1: Various Organisms isolated from vaginal discharge

Organism	No. of cases	Percentage
Gardnerella vaginalis	36	28.3%
Candida species	25	19.6%
Streptococcus pyogenes	16	12.5%
Trichomonas vaginalis	14	11.0%
Coagulase negative staphylococci	7	5.5%
Staphylococci aureus	5	3.9%
Enterobacteriaceae members - Klebsiella pneumoniae, Escherichia coli, Proteus mirabilis	7	5.5%
Pseudomonas	3	2.3%
Micrococci, Diptheroids, Aerobic spore bearers	8	6.2%
No growth	6	4.7%
Total	127	100%

Table-2: Correlation of Spiegel's criteria and culture in the detection of Gardnerella Vaginalis infection

PH>5	Amine test	Clue cells	Culture	NSV Cases	
				No	%
+	+	+	+	19	23.1
+	+	-	+	11	13.4
+	+	-	-	6	7.3
+	-	-	+	5	6.09
+	-	+	+	5	6.09
+	+	+	-	3	3.6
-	-	-	-	33	40.2
				82	100

Table No.3: According to Nugent's scoring system by Gram stain

Score	No of cases	Percentage
0-3	34	26.7%
4-6	64	50.3%
7-10	29	22.8%
Total	127	100

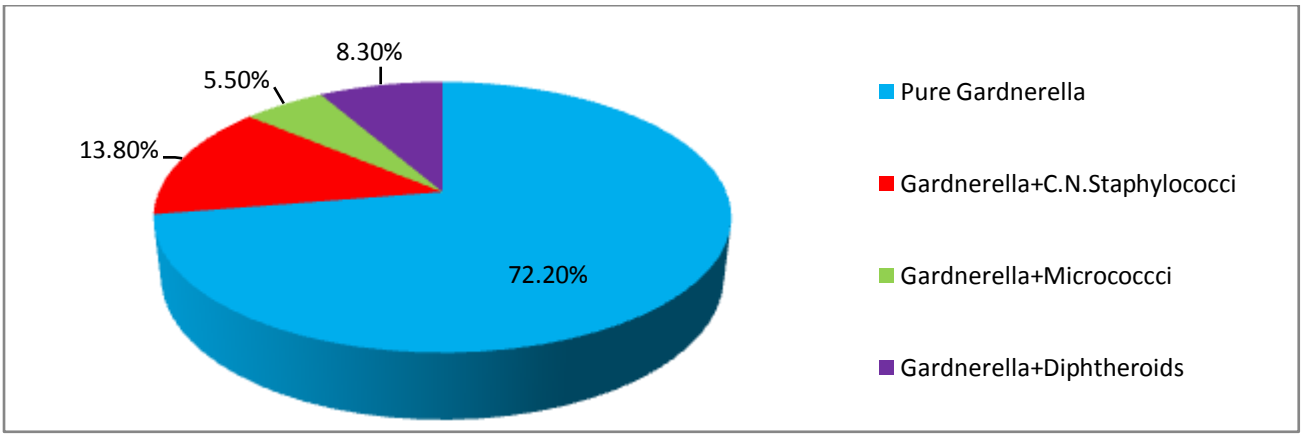

Fig-1: Association of Gardenerella vaginalis with other organisms

Table No.4: Antibiotic susceptibility testing of Gardnerella vaginalis

Drug	Drug in µg	sensitive	Percentage (%)
Ciprofloxacin	10	17	47.2
Ampicillin	25	25	69.4
Clindamycin	30	31	86.1
Co-trimaxozle	25	12	33.3
Metronidazole	50	34	94.4
Gentamicin	10	21	58.3

DISCUSSION:

Vaginal discharge is the common presenting symptom seen by gynaecologists. It is often a pathognomonic sign of underlying pathology, the commonest cause being infections.

In this study the incidence of vaginal infections increase with diabetes, of the 70 women studied 12.5% are diabetic. In Khawaja T Mahmood *et al.;* [11] study reported that vaginal infection rate was 71.4% among those with a past history of diabetes.

Gardnerella vaginalis was most common pathogen isolated in the present study about 28.3%. This result is in accordance with various authors Mirza NB *et al.;* [12] 75%, Leslie V H Hill [13] 68%, Soad Tabaq chali *et al.;* [14] 57%, Duttani IM *et al.;* [15] 46% and Vijaya D *et al.;* [16] 43.39%. Low incidence was reported by Dhall K *et al.;* [17] 12.8% and Pandit DV *et al.;* [18] 25.8%.

Isolation of Gardnerella vaginalis, majority were from the reproductive age group. This is in correlation with other worker Dhall K *et al.;* [17] who reported 75.6% incidence in the age group of 21-30.

In the present study it was observed that the isolates identified as Gardnerella vaginalis fulfilled the biochemical criteria like catalase test, oxidase test and fermentation of 1%glucose, 1%maltose and 1%starch. All 36 isolates were oxidase and catalase negative and fermented the sugars with acid only. Sarika Duggal *et al.;* [19] in their study have shown catalase, oxidase negative 100% and fermentation of maltose and starch 100%, fermentation of glucose 94.81% and hippurate hydrolysis 100%.

Vaginal pH was studied in all patients. Among 127 women studied, pH < 4.5 was noted in 34.6% and pH > 4.5 in 65.3%. The study correlates with V. Chandeying *et al.;* [20], who reported 57% of cases having PH >5. Amine test is specific for bacterial vaginosis, positive in 40.9%. This study correlates with studies done by Schaaf *et al.;* [21] who reported 32.5% cases of Amine test positive. On saline mount, 21.2% positive for clue cells in this study. The present study correlates with Abbott *et al.;* [22] and Schaaf *et al.;* [21] which show clue cells 31.5% and 32.5% respectively.

According to Nugent's score, 26.7% had normal flora with few commensals (score 0 – 3), 50.3% had intermediate score (4 – 6) where the smear should be correlated with clue cells count or repeat the test and 22.8% had bacterial vaginosis (score 7 – 10). The prevalence of bacterial vaginosis with score 7-10 in various studies like Abu Shaqra [23] was 29.7%, in Rouse *et al.;* [24] was 16.6% and in the study of Rizvi and Luby [25] was 25%.

An intermediate score (4 – 6) may be found among women who are either recovering from bacterial vaginosis or who may develop bacterial vaginosis subsequently. Such women therefore should be followed up to confirm the same as these alterations in vaginal micro flora may increase the risk of acquisition of other STIs including HIV infection.

In the present study the three tests used for diagnosis of NSV namely amine test, clue cells, and isolation in culture. It has found that all three were positive in only 19 out of 82 cases (23.1%), Amine test was positive and culture was negative in 9 cases (10.9%), amine test negative and culture positive in 10 cases (12.1%). This shows that amine test gives variable result so it is not used as a diagnostic tool for Bacterial vaginosis.

In our study among NSV cases correlation of clue cells and culture was found that 31 cases positive for clue cells 27 cases positive for culture, 18 cases positive for culture even in the absence of clue cells. In chronic cases due to the local Immunity IgA will destroy the clue cells. Similar observations were reported by Fule RP *et al.;* [26] had 33 culture positive with 27 clue cell positives, Meera Sharma *et al.;* [27] had 36 culture positives with 20 clue cell positives. In contrast to this study Mirza NB *et al.;* [12] (1983) had 100% correlation between clue cells and culture.

The commonest organism isolated in BV is Gardnerella vaginalis. Out of 36 isolates 26 (72.2%) were isolated in pure form and other 10(27.7%) were in association with others as mixed culture. The other organisms associated with Gardnerella vaginalis in mixed culture were coagulase negative staphylococci 5(13.8%), Micrococci 2(5.5%), and Diphtheroids 3 (8.3%). In this study there was no association of

Gardnerella vaginalis either with streptococci and Gram negative bacilli. The findings are in accordance with Fule RP *et al.;* [26] and Sarika Duggal *et al.;* [19]

Results of the antibiotic susceptibility of Gardnerella vaginalis revealed that the organism is most sensitive to Metronidazole (94.4%), followed by clindamycin (86.1%), Ampicillin (69.4%), gentamicin (58.3%), ciprofloxacin (47.2%) and Co-trimaxozle (33.3%). Study of invitro antibiotic susceptibility of Gardnerella vaginalis revealed metronidazole & clindamycin were the most sensitive drugs for Gardnerella vaginalis.

A similar observation was reported by Vastsala Dadhwal *et al.;* [28], Balsdon *et al.;* [29] and Pheifer *et al.;* [5]. Balsdon *et al.;* [29] and Pheifer *et al.;* [5] have confirmed that metronidazole therapy in a concentration of 500mg twice daily for 7 days was effective. Vastsala Dadhwal [28] has reported metronidazole was the most effective drug and can be used as a cream or gel as a local application. Bhujwala RA *et al.;* [30] have reported co-trimoxazole to be least sensitive.

Leucorrhoea is a common complaint in women of child bearing age. Women are less likely to seek treatment for the morbidity and thus are more likely to acquire other serious STI's. In view of this it is suggested that women attending antenatal and gynaecology clinics or family planning clinic should be screened and treated for BV cases to reduce risk of other STI.

CONCLUSION:

Ideal approach is the microbiological diagnostic approach for the aetiological diagnosis of symptomatic vaginal discharge such as simple microscopy, PH and Amine test with WHO algorithm has to be made before treatment. Health educational programmes through different media to educate women about the difference between normal and abnormal vaginal discharge and when to consult the gynaecologist.

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