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Non Specific Vaginitis with Special Reference to Gardenerella Vaginalis and Its Antibiotic Susceptibility Pattern

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ABSTRACT

Background: *Vaginal discharge, the major sign of vaginitis is the common reason for which women consult a Gynecologist. About 9 out of 10 patients with this problem suffer from infections of vagina. A symbiotic relationship between Gardnerella vaginalis and anaerobic bacterial flora, which changes vaginal ph is responsible for nonspecific vaginitis.*

Objective: *The present study was undertaken to study the Non specific vaginitis with special reference to Gardenerella vaginalis.*

Materials and methods: *200 women attending to Gynaecology op with the complaint of vaginal discharge of one week duration or more were selected as the study group along with a control group of 50 women attending the same op with complaints other than leucorrhoea. Sterile swabs were collected and transported in Amie's transport medium. Swabs were inoculated on to Columbia blood agar and incubated upto 48 hours in candle jar.*

Results: *G.vaginalis was isolated in 68(34%) of cases. The incidence was maximum in the age group of 21-30 years and women with greyish white discharge was 66.25%. Majority of isolates were sensitive to Ampicillin (51%) followed by metronidazole (50 µg) (36%).*

Conclusion: *our study findings are contrary to the belief. Therefore it is recommended that large sample based studies may be taken up in which may throw better light.*

Key words: *Gardenerella vaginalis, Vaginitis, Vaginal ph, Amies transport medium*

INTRODUCTION

Vaginal discharge, the major sign of vaginitis is the common reason for which women consult a gynaecologist. The most common cause of leucorrhoea is physiological, followed by vaginal infections due to bacteria, virus, fungi and parasites. Other causes include foreign bodies, cervicitis and atrophic vaginitis.[1] Vaginal discharge that, suddenly differs in colour, consistency and odour, and a significant increase or decrease in amount, may indicate an underlying abnormality of which infections are most common. The common conditions associated with abnormal vaginal discharge are bacterial vaginosis (50%), mycotic vulvovaginitis (20-25%) and trichimoniasis (15-20%).[2] Bacterial vagonosis 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 both aerobic and anaerobic flora.[3] Nonspecific vaginitis is abnormal discharge. altered vaginal ph, clue cells and absence of inflammatory response[4].The diagnosis of this condition is likely when a patient complains of a malodorous(fishy), non irrritating discharge and on examination reveals homogenous grey white secretions, but more than one half of patients with demonstrable signs have no symptoms[5] Malodor was due to volatilization of putrescine and cadaverine produced by vaginal anaerobic bacteria.[6] Vaginitis causes various pregnancy complications like preterm labour, premature rupture of membranes and chorioamnionitis[7,8]. 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[9]. 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 virginalis along with a control study of age matched women with no vaginal discharge. Antibiotic susceptibility pattern of Gardnerella vaginalis isolates also done.

MATERIALS AND METHODS

200 women attending to Gynaecological OP with the complaint of vaginal discharge of one week or more were selected as study group along with a control group of 50 women with complaints other than vaginal discharge. Samples were collected from OP of Gynaecology department in Narayana General Hospital, Nellore during January 2013 to June 2014.

A detailed Obstetrics and Gynaecology history was taken from all these women with special reference to vaginal discharge and its nature. General history of Diabetes, hypertension, use of oral pills and use of IUCD have been taken. The patients who gave the history of antimicrobial drug intake within 2 weeks or who have been found to have genital prolapsed, malignancy of the genital tract, or fibroid uterus were excluded from the study, Vaginal P^H was determined using P^H strips within a P^H range of 4 to 6 from the mid lateral wall with care taken to avoid contact with cervical mucus.

Two high vaginal swabs were collected with sterile swabs from posterior vaginal, fornix using a sterile sim's speculum. Swabs were transported to the microbiology laboratory in Amie's transport medium. Following sample collection speculum is withdrawn and sniff test was performed by the addition of 10% KOH onto the discharge collected on the speculum and the release of an intense fishy odour noted. Smear is prepared from the swab and gram staining was done and observed for presence of pus cells, clue cells, Gram negative coccobacilli, Gram negative cured rods (Mobiluncus). Smears were graded according to Nugent's scoring system.

Another swab was incoculated on to human blood agar with columbia blood agar base, Mac conkey agar, chocolate agar and blood agar plate was kept in the candle jar with a blotting paper to provide 5 to 10% CO2 and incubated for 48 hours. Culture plates were observed for diffuse β hemolytic tiny colonies on blood agar and no growth on Macconkey agar. Catalase and Oxidase tests are done from Chocolate agar and they were negative. Sugar fermentation test was done using 1% starch, 1% maltose and 1% glucose using phenol red as indicator.

Antibiotic susceptibility testing was done on human blood agar by Kirby – Bauer disc diffusion method using Metronidazole 10 µg, 25µg and 50µg, Ampicillin 25µg, gentamicin 10µg, ciprofloxacin 10µg, tetracycline 10µg streptomicin 10µg , kanamicin 30µg and cotrimoxazole 25µg.

RESULTS

200 women with vaginitis having increased malodorous homogenous vaginal discharge not attributable to conventional pathogens like Candida, Trichomonas vaginalis were diagnosed as having non- specific vaginitis.

The control group consisted of 50 women with matched age and without any complaints of vaginitis. Out of the 200 women majority of them were in the age group of 21 to 25(65) followed by women in the age group of 26-30(50). (Table-1).

Gardnerella vaginalis formed major isolate with 34% (68) .In the control group Gardnerella vaginalis was isolated in only 4% (2) of the cases. (Table-2)

In relationship of discharge with isolation of Gradnerella vaginalis in NSV Cases, Maximum number of cases were taken from Grey white discharge (80). Among them positive for G.vaginalis were 53 cases (66.25%) followed by purulent frothy discharge(45). Among them positive for G.vaginalis were 10 cases (22.22%). No isolation was seen among mucoid discharge. (Table-3)

Categorization of the smears in gram stain according to Nugent's scoring system revealed that maximum number of cases (46%) belonged to category ll with a score of 4 to 6 followed by category lll with a score of 68 (34%). Only few (20%) fell into category l with a score of 0-3. (Table-4)

Antimicrobial susceptibility testing showed most of the isolates were found to be sensitive to Ampicillin (51. 47%) followed by metronidazole (50 µg) (36.76%) . The organism was resistant to

metronidazole in the concentration of 10 µg and 25 µg. The sensitivity to other drugs was gentamicin (10µg) 36.76%, ciprofloxacin (10µg) 33.82%, tetracycline (10 µg) 25% , streptomycin (10µg) 22.05% and kanamycin (30 µg) 22.05% with least sensitivity to cotrimoxazole (25 mg) 7.3%. (Table-5)

Table: 1. Distribution of cases of Vaginitis Age – wise

Age in Groups	No of Cases	%	Controls No	%
16-20	35	17.5	10	20
21-25	65	32.5	11	22
26-30	50	25	16	32
31-35	28	14	6	12
36-40	14	7	3	6
41-45	5	2.5	3	6
46-50	3	1.5	1	2
Total	200	100	50	100

Table: 2 Incidence of Gradnerella vaginalis in NSV Cases and Control Group

CASES OF NSV			CONTROL		
No of cases studied	Positive for Gardnerella	%	No of cases studied	Positive for Gardnerella	%
200	68	34%	50	2	4

Table: 3 Relationship of discharge with isolation of Gradnerella vaginalis in NSV Cases

Type of discharge	No of cases	Isolation of G.Vaginalis	
		No	%
White Curdy	35	2	5.7
Purulent frothy	45	10	22.22
Grey White	80	53	66.25
Thin transparent	25	3	12
Mucoid	15	0	0
Total	200	68	

Table: 4 Categorisation of NSV cases according to Nugent's scoring system by Gram Stain

Category	Score	No of Cases	%
I	0-3	40	20
II	4-6	92	46
III	7-10	68	34
	Total	200	100

Table: 5 Antimicrobial susceptibility of G.Vaginalis from NSV cases

Drug	Conc in µg/disc	No. sensitive	%
Metronidazole	10	0	0
Metronidazole	25	0	0
Metronidazole	50	25	36.76
Ampicillin	25	35	51.47
Gentamicin	10	25	36.76
Ciprofloxacin	10	23	33.82
Co- Trimoxozole	25	5	7.35
Streptomycin	10	15	22.05
Kanamycin	30	15	22.05
Tetracycline	10	17	25

Nugent's scoring system for Gram- stained vaginal smears:

Organisms morphotype	Number/ oil immersion	Score
Lactobacillus like (Parallel sided Gram-positive rods)	>30	0
	5-30	1
	1-4	2
	<1	3
	0	4
Mobiluncus like (curved Gram – negative rods)	>5	2
	<1-4	1
	0	0
Garnerella/ Bacteroides like (Tiny Gram variable coccobacilli & rounded Pleomorphic, gram negative rods with vacuoles)	>30	4
	5-30	3
	1-4	2
	<1	1
	0	0

Total score is added up and interpreted as follows:

Score	Interpretation
0-3	Normal
4-6	Intemediate, repeat test later
7-10	Bacterial vaginosis

DISCUSSION

Irrespective of wealth of recent advances in diagnosis and treatment facilities symptoms of vaginitis form the major complaint of patients attending the Gynaecology outpatient department. The population in this study included patients complaining of varied amount of vaginal discharge of unspecified colour with or without inflammation of vaginal mucosa and existed for more than a week.

200 patients with vaginitis and matched control group of 50 women attending the Gynecology outpatient department were studied for evidence of presence of Gardnerella vaginalis to prove its association as a pathogen. Majority of the women were in the age group of 21 to 30 in the study as well age as control group. This study correlates with E.O.K. Nwankwo et al 2010, where maximum number was seen in age group 20-29 years.[11]

Although vaginitis caused by yeasts and Trichomonas vaginalis has been well described and documented other forms continue to present diagnostic challenge to both doctors and researchers. These forms come into the category of the commonly known entity of non specific vaginitis (NSV). Gardner and duke (1955) postulated the bacterial role of NSV which they called it Haemophilus vaginalis, later called as Gardnerella vaginalis. He himself isolated 92% of Gardnerella vaginalis [12]. Out of 200 cases 68 (34%) yielded positive isolation for Gardnerella vaginalis in pure or along with other isolates. Santosh saini et al(13) (1992) reported 18%, Rohtak and A.Black well (1982) reported 98% from London. Vijaya et al (14)(2000) from Bangalore reported an incidence of 43.39% which was closer to our study. The common co isolates were gram negative bacilli and some authors reported Staphylococci. It can be observed that the incidence of vaginitis due to Gardnerella vaginalis in India was comparatively low.

In the control group 4 (2%) were positive for Gardnerella vaginalis. But a higher incidence in control group was identified by Santosh saini et al (1992) 6%, Thakur et al (1986) 7.6% Sarika Duggal [15] (1992) 8, Bhuijwala (16)(1985) 16.66% and 1.m Dattani (1982)(17) 20%.

Ever since Gardener, when an attempt is made to correlate the 3 different tests used to diagnose NSV namely Amine test, clue cells and positive isolation in culture which will be gold standard for the diagnosis of bacterial vaginosis it was found that all 3 were positive only in 22% of cases, when Amine test was positive culture was negative in 9% where as in the presence of a negative Amine test there was a positive isolation in 8% cases. This shows that Amine test gives variable result and so cannot always be used as a clinical diagnostic tool for bacterial vaginosis.

In our study of correlation of clue cells positivity with positive isolation of Gardnerella vaginalis it was found that, out of 42 cases positive for clue cells 38 cases yielded positive isolation. On the contrary it was also found that in about 20% of cases even in the absence of clue cells there was positive culture. This can be explained on the basis of immunological response. In chronic cases due to local immunity Ig A will destroy clue cells. Similar observations by Santosh saini et al had 20

culture positive with 15 clue cell positives. R.P fule et al [18] had 33 culture positive with 27 clue cell positives. Meera Sharma et al [19] had 36 culture positives with 20 clue cell positives.

Nugent's scoring system [20] for gram stained vaginal smears has revealed that highest cases were in the category 2 and 3 score of 4 to6 fall in category of intermediate vaginal flora and those with a score >7 fall was diagnostic of bacterial vaginosis. It was observed from our study that Gardnerella vaginalis was the most common organism isolated in higher concentration in women suffering with NSV and the associated organisms were mostly commensal bacterial flora of female genital tract i.e., CN Staphylococci ,Micrococci, Diphtheroids.

All isolates identified as Gardenerella vaginalis were oxidase & catalase negative, fermented 1%glucose,maltose & starch with acid only. This is in correlation with Sarika duggal et al [21]

Study on the in vitro susceptibility of Gardnerella vaginalis revealed Ampiciilin as sensitive drug. A similar observation was reported by sarika Duggal, Thakur te al and Bhujala[22] et al. Bhujwala et al [22] reported cotrimoxazole as least sensitive.

The present study showed only 36.76% sensitivity to 50 µg disc of Metronidazole and resistance to 10 µg and 25µg , Sangeeta and Gill Amarjit kaur[23] have reported 33% sensitivity to 10µg, 46.7% to 25µg, 73.3% to 50 µg of Metronidazole . They have concluded that Metronidazole would be the drug of choice provided it is used in effective concentration.

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