INCIDENCE OF GARDNERELLA VAGINALIS IN NON-SPECIFIC VAGINITIS

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SUMMARY

The present study of incidence of Gardnerella Vaginalis in women with non-specific vaginitis included five hundred women with non-specific vaginitis and fifty age-matched women as controls. G. vaginalis was isolated in 232 (46.4%) cases. The incidence was maximum in the age group of 28-37 years. The maximum incidence of G. vaginalis was noted among the IUCD users i.e. 56.03% (Group 1), followed by pregnant women 22.41% (Group 2). The incidence in women complaining of pruritis vulvae was 15.52% (Group 3) and lastly the incidence of G. vaginalis amongst women presenting with backache and low abdominal discomfort was 6.04%(Group 4). Causative factors for G. vaginalis infection in the patients may be the tail of IUCD, hormonal effects of pregnancy and pelvic infalmmatory disease as a consequence of non-specific vaginitis.

Pure growth of G. vaginalis was obtained in 6.4% cases. Majority of isolates were sensitive to Amikacin, (90%), Erthyromycin (83%), Tetracycline (80%), Gentamycin (73%) Chloramphenicol (71%). Moderate sensitivity was shown with Kanamycin (69%), Cephalexin (62%), Ampicillin (50%), Ciprofloxacin (43%), Norfloxacin (40%) and Metronidazole 5 ug/disc. (26%).

INTRODUCTION

Vaginitis is a common gynaecological problem which may occur in three important clinical forms viz. Candial vaginitis, Trichomonal vaginitis and Non-specific vaginitis (NSV). Candial and Trichomonal forms have a definite aetiology, whereas

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NSV is controversial with regards to its actiology and therapy (Babu et al, 1987). It has alternatively been referred to as bacterial vaginosis (BV) which is not characterised by any inflammatory response. Bacteria which are commonly implicated with bacterial vaginosis are Gardnerella vaginalis, Bacteroides bivius and other bacteriodes species, Mycoplasma hominis and new species namely Mobiluncus mulieris and Mobiluncus curtisii (Mardh P.A, 1991).

G. vaginalis is now an accepted actioligical agent in the cases diagnosed as NSV. G. vaginalis is a small pleomorphic gram negative to gram variable bacillus measuring 0.3 to 2.3 x 0.5 in size. It is non-motile, non-capsulated, non-sporing, oxidase and catalase negative organism. It grows in culture from freshly collected samples of vaginal discharge and on media containing human blood (Hobbs B.C. et al, 1991).

MATERIAL AND METHODS

The present study was conducted in 500 patients attending the Out Patient Department (OPD) of Obst. & Gynae., Dayanand Medical College and Hospital, Ludhiana who were suffering from various types of clinical presentations associated with vaginal discharge suggestive of NSV.

Fifty healthy age-matched control women were also examined and studied in an identical manner. No subject from either group had any anti-microbial therapy before collection of samples. These patients were divided into four groups :-

Group I = Patients who were having intrauterine contraceptive device (IUCD) and were suffering from non-specific vaginal discharge.

Group II = Patients who were pregnant and having discharge.

Group III = Patients having discharge and pruritis vulvae.

Group IV = Patients having discharge associated with low backache/low abdominal discomfort. Samples of high vaginal swabs from these patients were investigated to determine the incidence of G. vaginalis.

COLLECTION AND TRANSPORT OF SAMPLES

Two samples of vaginal discharge were collected from diseased and control groups, using aseptic precautions in sterile swab tubes and brought to the laboratory within one hour.

PROCESSING OF SAMPLES

Samples were immediately processed as follows:-

a) Wet Smear Examination: Wet saline mount of vaginal discharge was examined under 40 x of microscope for presence of clue cells (vaginal epithelial cells with characteristic stippled or granulated appearence) pus cells, epithelial cells, yeast like cells and trichomonas vaginalis.

b) Aminetest : One drop of 10% potassium hydroxide was put on a clean glass slide in which a small portion of vaginal discharge was mixed. Production of fishy odour indicated positive amine test.

c) Gram smear : Smear was prepared on a glass slide, air-dried and heat-fixed and stained by Gram's method. The smear was examined under oil immersion lens of the microscope for the detection of various morphological forms of gram positive and gram negative organisms, clue cells, pus cells and yeast like cells etc. Detection of cells and small pleomorphlic gram negative

to gram variable bacilli (0.5 x 1.54) presumptively indicated presence of G vaginalis.

d) Culture : Specimens were inoculated on 5-10% citrated human blood agar (BA) made with Columbia agar base, Loeffler;s serum slope and MacConkey agar. Blood agar and Loeffler's serum slope were kept in a candle jar to provide 5-10% CO2. All the media, after inoculation, were incubated at 37°C and the plates were examined after 24, 48 and 72 hours of incubation.

IDENTIFICATION OF G. VAGINALIS

Minute opaque colonies with a narrow diffuse zone of Beta-haemolysis on Columbia blood agar were studied further by Gram's staining and biochemical tests. Growth, if any from Loeffler's serum slope was identified by gram's smear for G. vaginalis.

a) Catalase test.
b) Oxidase test.
c) Carbohydrate fermentation tests.
d) Hippurate hydrolysis (Piot P. et al, 1982).
e) Anti-microbial sensitivity pattern using modified Bauer & Kirby 1966) disc diffusion method. The various anti-microbial discs which were used for the sensitivity test were Ampicillin (10 mg.), Tetracycline (30 mg), Chloramphenicol (50 mg.), Kanamycin (30 mg.), Gentamycin (30 mg.), Metronidazole (5 mg.), Amikacin (30 mg.), Ciprofloxacin (5 mg.), Norfloxacin (10 mg.), Erythromycin (15 mg.) and Cephalaxcin (30 mg.).

OBSERVATIONS

The frequency distribution of different age group of patients and control group investigated for occurance of G. vaginalis is shown in (Table I).

Out of 500 cases, maximum number of patients were in the age group 28-37 years followed by age group 38-47 years respectively. The various types of vaginal discharge was distributed into four groups

Table I
AGE GROUP DISTRIBUTION OF NSV PATIENTS AND HEALTHY CONTROLS FOR G. VAGINALIS INVESTIGATIONS

Age group (year)	No. of patients		Control	
	Patients	Positive (%) (N = 500)	Patients	Positive (%) (N = 50)
18 - 27	120	40 (8%)	7	2 (4%)
28 - 37	235	162 (32.4%)	26	5 (10%)
38 - 47	145	30 (6%)	17	1 (2%)
	500	232 (46.4%)	50	8 (16%)

Table II

DISTRIBUTION OF NSV PATIENTS ACCORDING TO THEIR CLINICAL SYMPTOMS ASSOCIATED WITH VAGINAL DISCHARGE

Group	Description	No. of patients.	No. of culture positive case.	Pure growth
1.	Patients having IUCD associated with discharge.	240	130 (26%)	14(2.8%)
2.	Pregnancy associated with discharge.	100	52 (10.4%)	10 (2%)
3.	Vaginal discharge with pruritis.	104	36(7.2%)	06 (1.2%)
4.	Patients having backache/lower abdominal discomfort with discharge	56	14 (2.8%)	02 (6.4%)
	Total	500	232 (46.4%)	32 (13.8%)

Table III

OCCURRENCE PATTERN OF G. VAGINALIS IN ASSOCIATION WITH OTHER MICRO-ORGANISMS IN CULTURE OF VAGINAL SWABS FROM 500 NSV PATIENTS

Organisms associated with G. vaginalis	No. No. (200)	%
Esch. Coli.	38	19%
Klebsiella	36	18%
Strept. faecalis	33	16.5%
Proteus species	28	14.0%
Staphylococcus albus	27	13.5%
Candida species	38	19%
Total	200	40%

Total No. of cases positive for G. vaginalis - 232
Total No. of cases with pure growth of G. vaginalis - 32
Total No. of cases with mixed growth - 200

(Table II). There were 240 patients in group I who had IUCD associated with discharge and 130 were culture positive with 14 yielding pure growth. Of 100 patients in group II having pregnancy associated with discharge, 52 were culture positive, and of these, 10 yielded pure growth. Of 104 patients in group III who had vaginal discharge with pruritis, cultures yielded growth in 36, with showing pure growth and lastly, of the 56 patients complaining of backache, abdominal pain and disharge, growth was obtained in 2 cases in pure form out of 14 positive cases. A total of 232 (46.4%) cases yielded growth of G. vaginalis. Its occurence was highest (26%) in group I patients, followed by group II (10.4%), group III (7.2%), and minimal incidence of 2.8% among group IV patients.

Among control group, highest carrier rate was noticed among 28-37 years age group (10%) followed by 18-27 years (4%) & only 2% in age group of 38-47 years. Among 232 G. vaginalis culture positive cases, 32(13.8%) samples of vaginal discharge yielded pure growth. Another 200 cases of G. vaginalis culture showed mixed growth associated with various micro organisms (Table III). Most commonly associated organisms were Esch. coli and Candida species (19.0%) followed by Klebsiella 18% Strept. faecalis 16.5%, Proteus species 14.0% and Staphylococcus albus 13.5%.

The organism was identified on the basis of morphology in gram's smear and biochemical tests (Table IV). This organism is capable of hydrolysing Hippurate. However, due to non-availability of the reagents, this test could not be done in the present investigation. Antibiotic sensitivity of G. vaginalis isolates was performed on various antibiotics. The sensitivity to different drugs in descending order was Amikacin 90%, Erythromycin 83%, Tetracycline 80%, Gentamycin 73%, Chloramphenicol 71%, Kanamycin 69%, Cephalaxcin 62%, Ampicillin 50%, Ciprofloxacin 43%, Norfloxacin 40% and Metronidaxole 26% respectively.

Table IV

CRITERIA USED FOR IDENTIFICATION OF G. VAGINALIS

Test	Test indication positive reaction	
	No.	%age.
B Haemolysis (BH)	232	100
Glucose	232	100
Maltose	227	98
Lactose	0	0
Sucrose	14	6
Mannitol	0	0
Catalase	0	0
Oxidase	0	0

DISCUSSION

Non-specicic vaginitis is a very common and troublesome disease among all ages. Its signs and symptoms are variable and often not well defined. A large number of patients have been noticed as asymptomatic, hence diagnosis may be difficult and may be even missed.

Although, a wide variety of pathogens have been implicated in NSV, however, G. vaginalis has now been recognised as one of the leading causative agent of this problem.

Patients and healthy controls investigated in this study were belonging to reproductive age group i.e. 18 years to 47 years. Maximum number of patients (235) in study group and (26) in control group were between 28-37 of age. Similar findings have been reported by Saini et al (1992). IUCD was one of the commonest method of family planning, maximum number (240) patients belonged to this group followed by 104 cases of vaginal discharge with pruritis, while minimum number of patients (56) belonged to the fourth group.

G. vaginalis was isolated from 232 patients with NSV (46.4%) (Table II). In India, recovery rate of G. vaginais from patient with bacterial vaginitis varies from, 10.8 to 32.4% (Bhujwala et al, 1985; Deb et al, 1987 & Chandramukhi et al, 1981). However other workers isolated it in 41% of women with NSV (Levison et al, 1979). In control group, G. vaginalis was recovered from the vagina of 8 cases only. It is known that lactobacilli are part of normal flora of vagina that prevent the colonization of other pathogens in vagina by producing lactocins. This explains the low occurence of G. vaginalis among control group of women and higher incidence in patients with vaginal discharge.

In this study, the incidence of G. vaginalis was 46.4%. Maximum incidence of G. vaginalis i.e. 26% was in group I who were using IUCD. Rewari et al (1991) reported 79% among women who were using Cu-T200. It has been suggested that IUCD creates an anaerobic environment that increases the rate of NSV infection.

In 2nd group incidence of G. vaginalis was 10.4% Ray et al (1963) reported 20%. Patersen et al (1985) isolated G. vaginlis in 12% cases of pregnant women. In control group, Delaha et al (1969) found no difference in the incidence of G. vaginitis among pregnant & non pregnant women.

In 3rd group of study, the incidence of G. vaginalis was 7.2% which was higher than the incidence found in 4th group i.e. 2.8%.

Antibiogram pattern of different strains of G. vaginalis is rather diferent overall. The drugs which have shown good antimicrobial susceptibility invitro were Amikacin, Erthyromycin, Tetracycline, Gentamycin, Chloramphenicol, while Ciprofloxacin, Norfloxacin, Ampicillin were found to be of limited use invitro. Thus, the present study draws attention that sensitivity pattern is variable from strain to strain.

Metronidazole is considered to be the drug of choice of treatment of NSV patients. But in present study only 26% of G. vaginalis strains were susceptible. One reason for the higher resistance of agent may be the low concentration of the drug which was 5 ug/disc. used in the present study.

Pandit et al (1989) in a study isolated G. vaginalis from 71% cases of NSV and

found that none of the strains of G. vaginalis was sensitive to 5 ug/disc. of Metronidazole, whereas 93% of strains were sensitive to 50 ug. metronidazole disc. Bhujwala et al (1985) recorded highest sensitivity to Cholromphenicol (94.5%), Tetracycline (90.5%) followed by Erythromycin (86.4%) & Metronidazole 5 ug/disc. (59.1%).

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