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EPIDEMIOLOGICAL AND MICROBIOLOGICAL CORRELATES OF BACTERIAL VAGINOSIS

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Female population comprised of 544 with vaginitis and 258 asymptomatic were screened for presence of Bacterial Vaginosis (BV), Trichomonas vaginalis, Candida albicans, Gardnerella vaginalis and Mycoplasma hominis. Bacterial vaginosis, diagnosed on the basis of clinical criteria and gram's stained vaginal smear findings, was present in 50 percent of symptomatic cases and 21.8 percent of asymptomatic women. G. vaginalis, M. hominis and T. vaginalis were significantly associated with a diagnosis of BV. BV showed a positive correlation with lower socio-economic status and parity of more than 2. Prevalence of G. vaginalis was significantly higher among women who were ≥33 years of age, had been married for a shorter duration, gave history of intercourse in the preceding 48 hours and were of lower socio-economic status.

Key Words : Bacterial vaginosis, Epidemiology, G. vaginalis

Introduction

Bacterial vaginosis (BV) is reported to be the commonest form of vaginitis in gynaecologic outpatients and STD attenders, yet there is very little reliable data on the epidemiology of this syndrome. A major limitation in most studies on BV has been the lack of clearly defined study groups, since many investigators have not applied the diagnostic criteria put forth by Gardner and Dukes[1] and re-evaluated by Amsel et al.[2]

Mobiluncus species, Gardnerella vaginalis and Mycoplasma hominis are independently associated with a clinical diagnosis of BV.[3] Due to the close association of G. vaginalis with BV, many earlier investigators tended to rely solely on the presence or absence of this organism to diagnose BV and therefore focused on G. vaginalis associated vaginitis rather than on BV as such. Several studies have investigated the epidemiology of G. vaginalis and found an association with race, previous pregnancy, oral contraceptives and sexually transmitted diseases.[4-7]

The epidemiology of BV, diagnosed on the basis of reliable clinical criteria, has been studied.[2,7,8] A significant positive correlation has been reported with increasing age, history of sexual activity, previous trichomoniasis, use of intrauterine device, other co-existant infection due to Neisseria gonorrhoea, Chlamydia trachomatis and inflammatory report in wet smear.

The purpose of this study was to determine the prevalence, the microbiological and epidemiological correlates of BV among symptomatic and asymptomatic women attending the gynaecologic and Family Welfare clinics of a large city hospital.

Materials and Methods

This study was conducted from January 1988 to December 1990 and comprised of 544 women with symptoms of excessive vaginal discharge with or without pruritis and

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malodour and 258 asymptomatic women. Women who were menstruating or had received any treatment for vaginitis in the preceding six weeks were excluded. Detailed history was obtained from each patient about socio-economic status, present genital complaints, age, martial status, parity, stage of menstrual cycle and hours since last intercourse.

Each patient was subjected to per speculum examination to note the source, amount and type of vaginal discharge present. Women showing evidence of mucopurulent cervical discharge, cervical erythema or friability were excluded.

High vaginal swabs and cervical swabs were collected and transported to the laboratory for microscopy and culture. A bimanual per vaginum examination was done to rule out any palpable pelvic pathology.

The pH of the vaginal secretions was measured and Amine test was done using 10% KOH.[2]

Wet mount smear and Gram stained smear of the vaginal secretions were made and examined. Large gram positive bacilli (Lactobacillus morphotype) and small gram negative or gram variable coccobacilli (Gardnerella morphotype) were estimated semi-quantitatively according to the following scheme : 1-10/field - scanty, 10-30/field - moderate and >30/field - predominant.[9]

Bacterial vaginosis was diagnosed when 4 of the following 6 criteria were fulfilled.[2,9]

a. Presence of a homogenous, thin vaginal discharge

b. Vaginal pH higher than 4.5

c. Positive Amine test

d. Presence of clue cells

e. Absence or scarcity of lactobacillus morphotype and

f. Predominance of small gram negative or gram variable coccobacilli (Gardnerella morphotype).

Vaginal specimens were collected for detection of G. vaginalis, T. vaginalis and C. albicans by microscopy and culture.

Cervical specimens were obtained for detection of N. gonorrhoea and M. hominis infection.

Culture Methods

G vaginalis : Vaginal specimens were inoculated on human blood bilayer medium[10] and Columbia agar with 5% human blood[11] and incubated at 37°C in 5-10% CO_2 for 48-72 hours. Isolates that produced tiny transparent colonies with diffuse beta hemolysis, were gram negative or gram variable short rods and were oxidase and catalase negative were presumptively identified as G. vaginalis. Further tests that were put up for confirmation included hydrolysis of starch and hippurate, presence of α-glucosidase and absence of β-glucosidase.[11]

M hominis : Cervical specimens were transported in Mycoplasma Collection Broth, inoculated into Arginine Broth with erythromycin (AE_{100} Broth) and the pH measured daily during incubation for 11 days. Broths which showed a rise in pH were subcultured to AE_{100} agar that were incubated at 37°C in 5-10% CO_2 and examined after 2-4 days. Colonies of M. hominis were identified by their characteristic morphology and by inhibition of growth by specific antiserum.[12]

N. gonorrhoea : Cervical specimens were directly inoculated on selective

chocolate agar (with VCN supplement) for culture of N. gonorrhoea.[13]

C. albicans : Culture for C. albicans was performed on Sabouraud's Dextrose Agar.[13]

T. vaginalis : Culture for T. vaginalis was performed on modified Diamond's medium.[14]

Results

In the present study 544 women with symptoms of vaginitis and 258 asymptomatic women were screened for presence of BV on the basis of the well established clinical criteria[2] and interpretation of gram stained vaginal smear[10]. Among cases, BV was detected in 50%, while amongst asymptomatic women BV was found in 21.8%. The prevalence of various genital pathogens associated with vaginitis including BV in the various clinical groups of patients is shown in Table I. G. vaginalis, T. vaginalis and M. hominis were found to be significantly associated with BV as compared to healthy asymptomatic controls.

Table II shows the epidemiological risk factors associated with BV. A longer duration of sexual activity as indicated by duration of marital status was significantly associated with BV ($p<0.05$). A lower socio-economic status and parity of more than 2 were also risk factors for development of BV ($p<0.01$).

Although G. vaginalis was significantly correlated with BV, the epidemiological correlates of G. vaginalis colonization were not similar to those of BV. Prevalence of G. vaginalis was significantly higher among women who were less than or equal to 33

Table I. Prevalence of various genital pathogens in cases and controls.

Patient group	No.	No.(%) showing presence of			No. of samples processed for M. hominis	No.(%) positive for M. hominis
		G. vaginalis	C. albicans	T. vaginalis		
Symptomatic						
Bacterial vaginosis	272	116* (42.6)	20 (7.3)	24** (8.8)	230	26** (11.3)
Other vaginitis	272	48 (17.6)	18 (6.6)	6 (2.2)	216	14 (6.4)
Asymptomatic						
Bacterial vaginosis	56	21 (37.5)	2 (3.5)	3 (5.3)	44	2 (4.54)
Healthy controls	202	51 (25.2)	22 (10.8)	2 (0.99)	182	8 (4.39)
Total	802	236	62	35	672	50

* $p =< 0.001$ as compared to healthy controls
** $p =< 0.05$

years of age and had been married for less than 6 years. G. vaginalis was recovered more frequently from women who gave a history of intercourse within the preceding 48 hours ($x^2 = 3.55$) and who were of lower socio-economic status ($x^2 = 3.63$), but these findings were not statistically significant (Table III).

Discussion

The prevalence of BV varies with the population studies and the criteria employed for diagnosis. Using objective clinical criteria and interpretation of gram stained vaginal smears for the diagnosis of BV; we found a prevalence of 50% among symptomatic and 21.7% among asymptomatic women. BV was clinically diagnosed in 27.2% of symptomatic women in a study carried out in France[7] and in 25% of unselected consecutive females presenting at a students health centre gynaecology clinic in Washington,[2] half of them being asymptomatic. A similar prevalence rate has been reported even among STD attenders in Sweden.[8] All the above three studies employed objective criteria for the diagnosis of BV. We found a higher prevalence rate

Table II. Relationship between BV and various epidemiological factors.

Epidemiological factors	n=802	No. (%) positive for BV	x^2	P
Age				
≤33 years	611	247 (40.4)	0.236	NS
> 33 years	191	81 (42.4)		
Marital Status				
≤6 years	244	84 (34.4)	6.07	< 0.05
> 6 years	558	244 (43.7)		
Parity				
≤2	487	173 (35.5)	14.8	< 0.001
> 2	315	155 (49.2)		
Stage of Menstrual Cycle				
Pre-ovulatory	362	147 (40.6)	0.02	NS
Post-ovulatory	440	181 (41.1)		
Last Coitus within				
≤48 hours	231	95 (41.1)	0.006	NS
> 48 hours	571	233 (40.8)		
Socio-Economic Status				
Upper/Upper Middle	330	116 (35.1)	7.65	< 0.01
Lower Middle/Upper Lower	472	212 (44.9)		

NS - not significant.

among gynaecologic outpatients possibly because all of them were sexually active married women, largely from a lower socio-economic status.

G. vaginalis and M. hominis have been correlated with BV in several studies[7,8,15] although the exact pathogenetic mechanisms involved are yet unknown. In the present study also both these organisms were significantly associated with symptomatic BV.

T. vaginalis and C. albicans could be detected in only 5.5% and 6.9% of symptomatic women respectively in our study. T. vaginalis was also significantly associated with symptomatic BV. Earlier studies have also revealed a significant association between a past history of trichomoniasis and BV and 80% of women infected with T. vaginalis could be diagnosed as BV, particularly symptomatic women and

Table III. Relationship between colonisation with G.vaginalis and various epidemiological factors.

Epidemiological factors	n=802	No. (%) positive for G. vàginalis	x^2	P
Age				
≤33 years	611	197 (32.2)	9.79	< 0.01
> 33 years	191	39 (20.4)		
Marital Status				
≤6 years	244	90 (36.8)	9.39	< 0.01
> 6 years	558	146 (24.8)		
Parity				
≤2	487	147 (30.1)	0.34	NS
> 2	315	89 (28.2)		
Stage of Menstrual Cycle				
Pre-ovulatory	362	113 (31.2)	1.01	NS
Post-ovulatory	440	123 (27.9)		
Last Coitus within				
≤48 hours	231	79 (34.1)	3.55	NS
> 48 hours	571	157 (27.4)		
Socio-Economic Status				
Upper/Upper Middle	330	85 (25.7)	3.63	NS
Lower Middle/Upper Lower	472	151 (31.9)		

NS - not significant.

women attending STD clinics.[2,16] However, since mixed vaginal infections due to T. vaginalis and BV associated microorganisms occur frequently, no etiological relationship can be derived from any statistically significant association between presence of T. vaginalis and BV.

A clinical diagnosis of BV in our study population was significantly correlated with increasing years since marriage, a lower socio-economic status and a parity of more than two but not with age, stage of menstrual cycle and hours since last intercourse. Amsel et al[2] also did not find any association between BV (non-specific vaginitis) and age, stage of menstrual cycle or age at menarche, while the proportion of patients with a history of previous pregnancy, previous abortion, mean number of pregnancies and years of sexual activity were all higher among patients with nonspecific vaginitis than among normal subjects. A significant negative correlation of BV with barrier contraceptives, genital warts and vulvovaginal candidiasis has also been observed.[8]

The epidemilogical correlates of G. vaginalis also vary with the population studied. In an earlier study, colonization with G. vaginalis was more frequent among women with low socio-economic status, who were non-pregnant, in the post-ovulatory stage of menstrual cycle and using non-protective contraceptives.[6] In another study, logit analysis defined four factors that were significantly associated with colonization with G. vaginalis : nonwhite race, use of oral contraceptives, no history of marriage and history of pregnancy.[4] The prevalence of G. vaginalis is not reported to be influenced by age,[4,6,7] total number of sexual partners, day of menstrual cycle or method of contraception[4]. We found that G. vaginalis colonization was significantly associated with age (less than 33 years) and marital status (duration less than 6 years) but not with parity or stage of menstrual cycle.

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