Research Article

A Comparative study of prevalence of bacterial vaginosis in HIV positive women and HIV seronegative consorts of HIV positive males

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Abstract

Aim and Objectives: To study prevalence of bacterial vaginosis in HIV seropositive females, Consorts of HIV positive males & control group and association of other reproductive tract infections with bacterial vaginosis and assessment of progression of HIV infection in bacterial vaginosis positive patients by CD 4 cell count.

Method: This prospective randomized study was carried out at STD Clinic, Zenana Hospital with approval from Hospital Ethics Committee and informed written consent from patients.

50 HIV seropositive women, 25 seronegative consorts of HIV positive males and 50 control patients were included in the study. All the patients under study were subjected to Grams staining of the vaginal smear, Pap smear and Colposcopy. HPV DNA testing was done in all HIV positive patients and seronegative consorts of HIV positive males. In control group only patients showing dyskaryotic changes on cytology were subjected to colposcopy. All HIV seropositive women were subjected to additional tests of CD 4 and CD 8 cell counts.

Result: Prevalence of bacterial vaginosis was high in HIV positive women (36%) as compared to HIV negative group (24%). Prevalence was significantly higher in HIV positive patients with CD 4 cell count less than 200 per microlitre that is 77.77 % with P value (0.007) which is highly significant statistically.

Conclusion: In bacterial vaginosis, vaginal eco-system is altered. It increases risk of infection by opportunistic pathogens when the host defences became impaired. Hence it is concluded that bacterial vaginosis should be treated effectively in patients who are at high risk of acquiring HIV infection and wide spread control of bacterial vaginosis can become a tool for decreasing incidence of HIV in developing countries.

Keywords: Bacterial vaginosis, HIV, CD4 cell count, Colposcopy, Gram stain

1. Introduction

Vagina has a dynamic ecosystem. The vaginal pH in premenarchal female is 7. At the time of puberty under the influence of estrogen, the vaginal epithelium increases to 25 cell thickness with increased glycogen levels and the predominant flora changes to lactobacilli and vaginal ph is less than 4.5 due to production of lactic acid. This low pH is maintained till menopause.

Bacterial Vaginosis is a polymicrobial syndrome involving replacement of normal vaginal lactobacilli with concentration of Gardnerella vaginalis, Mobilincus species, Mycohominis, Anaerobic gram negative rod belonging to Prevotella, Porphyromonas, Bacteroids and Peptostreptococcus species.

Gardenerella vaginalis has been found in 45% to 98%, Anaerobic gram negative rod in 53% to 76%, Peptostreptococcus in 29% to 63%, Mycohominis in 58% to 76% and Mobilincus in 51% of Bacterial Vaginosis cases and
0% in controls; Whereas lactobacilli have generally been found in 9% to 38% of Bacterial vaginosis cases and 68% to 90% of controls.\(^1\)

Prevalence ranges from 5% for women without any symptom to 25% for those with gynecologic symptoms.\(^2\) Upto 50% of women are asymptomatic.\(^3,4\)

Bacterial vaginosis is a common gynecologic infection that has been associated with a variety of gynecologic and obstetric complications, including pelvic inflammatory disease\(^5,6\), postabortal infection, Chorioamnionitis\(^7\), Preterm delivery, tubal infertility, postpartum endometritis, CIN\(^4\) and HPV infection. Recent studies suggest that bacterial vaginosis may increase a woman’s risk for human immunodeficiency virus (HIV). As the proportion of women acquiring HIV through heterosexual transmission continues to increase, emerging research demonstrates an association between the presence of bacterial vaginosis and increased susceptibility to HIV.

The presence of bacterial lysates have been shown to stimulate HIV expression in in-vitro monocytoid and certain T cell lines.\(^8\) A recent study in Chicago collecting cervicovaginal lavage specimens found Gram stain indicative of bacterial vaginosis to be significantly associated with a newly identified HIV inducing factor that induces HIV 1 gene expression.\(^9\)

The potential mechanism to explain the association of bacterial vaginosis and HIV include:

1. Peroxide producing lactobacilli predominate in normal vaginal flora and maintain a low pH.
2. Peroxide producing lactobacilli demonstrated viricidal effects on HIV-1.\(^12\)
3. Combining peroxidases and halide ion (both present in the vagina) with peroxide enhances the viricidal activity from 51% to nearly 100%.
4. A low vaginal pH inhibits CD4 + lymphocyte activation and may reduce the number of target cells for HIV -1 in the vagina.\(^13\)

An association of HIV infection and bacterial vaginosis has been reported in both cross sectional and longitudinal studies. In the cross sectional studies, women with bacterial vaginosis had a higher prevalence of HIV infection. Studies carried out in high HIV-1 prevalent populations in Africa have found BV to be more prevalent among women who are HIV positive. A study in Uganda reported an odds ratio for HIV-1 of 2.08(95 %,CI 1.48-2.94 )in women with severe BV ( score of 9-10 on Grams stain)compared with those with normal vaginal flora.\(^14,15\)

Prevalence studies show that there is potentially large reservoir of BV in the population. At present BV can be definitely linked to increased risk of HIV-1 acquisition.

2. Materials and method

This study was conducted in 50 HIV positive women (Group A) ,25 HIV seronegative consorts of HIV positive males (Group B) and 50 control patients (Group C) at STD Clinic Zenana hospital in collaboration with STD department, SMS Medical College.

2.1 Inclusion criteria

1. Diagnosed cases of HIV and consorts of HIV positive males.
2. Women in reproductive age group i.e between 20-45.
3. Women with complaints of vaginal discharge, pain lower abdomen, burning, itching vulva , dyspareunia , backache.
4. Women having multiple sexual partners.

2.2 Exclusion Criteria:

1. Any patient with bleeding per vaginum.
2. Pregnant females.
3. Postmenopausal females.
4. Diagnosed cases of Carcinoma Cervix.
5. Patients having Prolapse Uterus.
6. Patients who have used vaginal pessary or douche in last 4-5 days.

All the patients were subjected to detailed clinical history on predesigned proforma, Examination, Pap’s smear, Grams smear of vagina, Colposcopy, HPV DNA testing, HIV ELISA and CD 4/CD8 ratio in HIV positive patients.

For diagnosis of bacterial vaginosis two approaches were used

- Amsel criteria\(^10\). At least three of the four criteria should be present for the diagnosis to be confirmed.
  1. Thin, white, homogeneous discharge
  2. Clue cells on microscopy of wet mount
  3. pH of vaginal fluid >4.5
  4. Release of a fishy odour on adding alkali (10% KOH).
• A Gram stained vaginal smear, evaluated with the Nugent criteria\textsuperscript{11}.

The Nugent score is derived from estimating the relative proportions of bacterial morphotypes to give a score between 0 and 10. A score of <4 is normal, 4-6 is intermediate, and >6 is BV\textsuperscript{11}.

All study patients were subjected to Pap smear. Colposcopy and HPV DNA testing were done in all HIV positive patients and seronegative consorts of HIV positive males and in control group only patients showing dyskaryotic changes on cytology were subjected to colposcopy.

All HIV seropositive women were subjected to additional tests of CD 4 and CD 8 cell counts.

3. Results

Maximum number of patients were in the age group of 21 to 30 years with 60% in HIV positive (Group A), 80% in consort group (Group B) and 44% in the control group (Group C). It shows that most of the women were sexually active. (Table 1)

| Age (in yrs) | Group A HIV positive women (n=50) | Group B Consort of HIV positive males (n=25) | Group C Control group (n=50) |
|-------------|-------------------------------|---------------------------------|----------------------------|
|             | No. | %    | No. | %    | No. | %    |
| <20         | 2   | 4    | 2   | 8    | 6   | 12   |
| 21-30       | 30  | 60   | 20  | 80   | 22  | 44   |
| 31-40       | 15  | 30   | 3   | 12   | 17  | 34   |
| >40         | 3   | 6    | -   | -    | 5   | 10   |
| Total       | 50  | 100  | 25  | 100  | 50  | 100  |

In HIV positive patients 18 i.e. 36%, in consort group 8 i.e. 32% and in control group 12 i.e. 24% patients had Bacterial Vaginosis. (Table 2)

| Prevalence of Bacterial Vaginosis | Group A HIV positive women (n=50) | Group B Consort of HIV positive males (n=25) | Group C Control group (n=50) |
|----------------------------------|----------------------------------|---------------------------------|----------------------------|
|                                  | No. | %    | No. | %    | No. | %    |
| Bacterial Vaginosis positive     | 18  | 36   | 8   | 32   | 12  | 24   |

In our study it was found that in HIV positive group Herpes (38.85%) was most prevalent infection followed by Candidiasis (33.33%) and Trichomoniasis (33.33%). In Consort and control group Trichomoniasis was most prevalent i.e. 16% and 24% respectively. (Table 3)

| RTIs and STIs                  | Group A HIV positive women (n=50) | Group B Consort of HIV positive males (n=25) | Group C Control group (n=50) |
|--------------------------------|-----------------------------------|---------------------------------|----------------------------|
|                                 | BV+ | BV- | BV+ | BV- | BV+ | BV- |
|                                 | No. | %   | No. | %   | No. | %   |
| Candidiasis                    | 6   | 33.33 | 8  | 25  | 1   | 12.5 | 1  | 5.88 | 2  | 16  | 8  | 21  |
| Trichomoniasis                | 6   | 33.33 | 8  | 25  | 2   | 25   | 2  | 11.7 | 6  | 43  | 6  | 15  |
| HPV                            | 4   | 22.22 | 3  | 9.36 | -  | -    | -  | -   | 1  | 8.3 | -  | -   |
| Gonorrhea                      | 1   | 5.55  | 2  | 6.24 | -  | -    | -  | -   | 1  | 8.3 | 2  | 5.2 |
| Chlamydia                      | 3   | 16.66 | 1  | 3.12 | 2  | 25   | -  | -   | 3  | 25  | 2  | 5.2 |
| Syphilis                       | 1   | 5.55  | 2  | 6.24 | -  | -    | -  | -   | -  | 1   | 2.6 |
| Herpes                         | 7   | 38.85 | 8  | 25  | -  | -    | -  | -   | 1  | 5.88 | 2  | 16  |
| Hepatitis B                    | -   | -    | 1  | 3.12 | -  | -    | -  | -   | -  | -   | -  | -   |
| Scabies                        | 2   | 11.10 | -  | -    | -  | -    | -  | -   | -  | -   | -  | -   |
According to cytology- maximum no. of patients in HIV, consort and control group had inflammatory smear with 58% (i.e 29 cases) in HIV positive, 68% (i.e 17 cases) in consort group, and 54% (i.e 28 cases) in control group. Koilocytic /dyskaryotic changes were seen in 14% in group A and 6% in group C. (Table 4)

In bacterial Vaginosis positive cases, 50% of HIV positive females that is 9 cases presented with vaginal discharge with multiple symptoms. 62.5%of patients in consort group presented with vaginal discharge only. While in control group 50% of patients presented with vaginal discharge and pain in abdomen.

According to the colposcopic findings, out of 18 bacterial vaginosis positive HIV positive patients 55.55 % had no abnormality. Grade I, II and III changes were seen in 16.66, 16.66 and 11.11 % of HIV positive patients. (Table 5)

| Table 4 Distribution of patients according to cytology |
|--------------------------------------------------------|
| **Cytology Findings** | **Group A HIV positive women(n=50)** | **Group B Consort of HIV positive males(n=25)** | **Group C Control group (n=50)** |
| | BV+ | BV- | BV+ | BV- | BV+ | BV- | BV+ | BV- |
| NAD | 2 | 22 | 10 | 31 | 3 | 3.75 | 5 | 29.91 | 2 | 16.66 | 17 | 44 |
| Inflammatory | 9 | 50 | 20 | 62 | 5 | 62.5 | 12 | 70.58 | 10 | 83.33 | 18 | 47 |
| Koilocytic/Dyskaryotic | 5 | 27 | 2 | 6.2 | - | - | - | - | - | - | 3 | 7.8 |
| Total | 18 | 100 | 32 | 100 | 8 | 100 | 17 | 100 | 12 | 100 | 38 | 100 |

The high prevalence of Koilocytosis and Dyskaryosis was probably due to high prevalence of HPV (22.22%) infection in HIV positive group. In control group only 3 patients who showed dyskaryotic changes on cytology were subjected to colposcopy. Out of 3, one had Grade I changes and two cases had Grade II changes.

According to CD4 count – in HIV positive group , patients having CD4 cell count <200/MicroL showed high prevalence of bacterial vaginosis i.e 77.77% showing a high association between decreased CD4 cell count and acquisition of bacterial vaginosis. (Table 6)

| Table 5. Distribution of patients according to Colposcopic findings |
|---------------------------------------------------------------|
| **Colposcopic findings** | **Group A HIV positive women (n=50)** | **Group B Consort of HIV positive males (n=25)** | **Group C Control group (n=50)** |
| | BV+ | BV- | BV+ | BV- | BV+ | BV- | BV+ | BV- |
| NAD | 10 | 55.5 | 15 | 46 | 6 | 75 | 14 | 82.3 | - | - | - | - |
| Grade I | 3 | 16.6 | 14 | 43 | 2 | 25 | 3 | 17.6 | 1 | 100 | - | - |
| Grade II | 3 | 16.6 | 2 | 6.2 | - | - | - | - | - | - | 2 | 100 |
| Grade III | 2 | 11.1 | 1 | 3.1 | - | - | - | - | - | - | - | - |
| Total | 18 | 100 | 32 | 100 | 8 | 100 | 17 | 100 | 1 | 100 | 2 | 100 |

| Table 6 Distribution of patients according to Absolute CD4 cell count |
|---------------------------------------------------------------|
| **Absolute CD4 cell count** | **Group A HIV positive women(n=50)** | **Group B Consort of HIV positive males(n=25)** |
| | BV+ | BV- | BV+ | BV- | BV+ | BV- |
| >500 | - | - | 7 | 21.87 | 8 | 100 | 17 | 100 |
| 200-499 | 4 | 22.22 | 14 | 43.75 | - | - | - | - |
| <200 | 14 | 77.77 | 11 | 34.37 | - | - | - | - |
| Total | 18 | 100 | 32 | 100 | 8 | 100 | 17 | 100 |
4. Discussion

In our study, we observed that prevalence of bacterial vaginosis was more in HIV positive women as compared to Consort and control group. Cohen et al noted a significant association of Bacterial Vaginosis and HIV seropositivity (OR, 2.7; 95% CI 1.3-5) in female sex workers in Thailand\textsuperscript{18}. Sewankambo et al and co workers in Raki area of Uganda, reported that women with Bacterial Vaginosis were at increased risk of HIV infection ( OR 1.5). Studies carried out in high HIV -1 prevalence population in Africa have found Bacterial Vaginosis to be more prevalent among women who are HIV positive.\textsuperscript{15}

In a study by Warren et al on 854 HIV positive and 434 HIV negative women, it was found that prevalence of Bacterial Vaginosis was 47 % in HIV positive as compared to 44% in HIV negative women ; this difference was not statiscally significant ( P=0.36).\textsuperscript{19}

In present study HIV positive patients showed more prevalence of Candidiasis (28%), Trichomoniasis (28%), Herpes ( 30%) followed by HPV (14%)and Chlamydia (8%). But in HIV positive patients with Bacterial Vaginosis prevalence of above infections was more i.e Candidiasis (33.33%) , Trichomoniasis (33%) , Herpes (38.85%), HPV (22.22%).In consort and control group patients with Bacterial Vaginosis most common co- infection was of Trichomoniais.This correlate well with study done in STD clinic in Seattle in which 75% of women with trichomoniasi also had Bacterial Vaginosis compared with 47% of women without Trichomoniais ( P <0.001).\textsuperscript{20}

Cu Uvin et al in his study found a prevalence of Trichomoniasis in 9.4 % - 29.5% of HIV positive and 8.2% - 23.4% of HIV negative females.\textsuperscript{21} Andrew Helfgott et al found that difference of frequency of prevalence of Trichomoniasis in HIV positive and HIV negative group was significant ( P=0.015,OR, 9.5;95%CI, 1.6%,54.9%).HPV was prevalent in 8.4% of HIV positive and 7.1% of HIV negative women. HIV was prevalent in 8.2% of HIV positive and 0% in control group (P=0.014).Study showed a high prevalence of Chlamydia in HIV negative ( P<0.0001).\textsuperscript{16}

Minkoff et al reported that HIV positive were 3.4 times more likely to have HPV infection as compared to general population ( P<0.001 ,95% CI, 3.13%-4.88%).\textsuperscript{22}

Landers et al in a study of 598 women reported Bacterial Vaginosis in 276 (46%), vaginal yeast (29%), Trichonomiasis (12%) and Chlamydia (11%).The most prevalent co-infection was Bacterial vaginosis with Trichonomiasis.\textsuperscript{17}

In our study in all the 3 study group cytology showed Inflammatory smear in maximum no. of cases i.e HIV positive(58% )consort ( 68%), and control group (54%). 7 patients (14%) in HIV positive and 3 cases(6%) in control group had koilocytosis and dyskaryotic changes. Out of 7 cases in HIV group, 5 patients were Bacterial Vaginosis positive. This high prevalence was probably due to high prevalence of HPV (22.22%) infection in HIV positive females with Bacterial Vaginosis. This correlates with a retrospective study done in Sweden, CIN was significantly more common among women with Bacterial Vaginosis.\textsuperscript{23}

We observed a strong correlation between Bacterial Vaginosis and CD4 cell count. Prevalence of Bacterial vaginosis in HIV positive patients with CD4 cell count < 200 / Microl was 77.77% in our study which is highly significant statistically with P=0.007. Further studies are mandatory to potentiate these findings.

5. Conclusion

The overall evidence suggests that in Bacterial vaginosis vaginal ecosystem is altered; it increases risk of infection by opportunistic pathogens when the host defences become impaired.

Hence it is concluded that BV should be effectively treated in patients who are at high risk of acquiring HIV infection & wide spread control of BV can become a means for decreasing incidence of HIV in developing world.

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