

Etiology of Symptomatic Vaginitis among HIV/AIDS Patients in the Era of Highly Active Antiretroviral Therapy

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Abstract

Background: HIV/AIDS is a widespread public health problem in India and rest of the World, causing severe morbidity and mortality. Occurrences of opportunistic infections (OI's) in HIV/AIDS correlate with their immune status. Infectious vaginitis is commonly caused by bacteria, fungi, and parasites. In general, the vaginal acidity limits the development of these infections, but the risk increases with a decrease in the immune status. Vaginitis is the most common and important OI in HIV seropositive women. The cause of concern is the recurrent and multiple episodes of vaginitis, and the emergence of drug resistance. In the general population, almost 70% of women experience at least one episode of vaginitis in their lifetime, making the condition even worse in HIV infected women resulting in recurrent and chronic infections. Knowledge of vaginitis in HIV seropositive women is inadequate. **Aim:** Hence this study aims to evaluate the etiology of vaginitis among HIV seropositive women before and after initiation of highly active antiretroviral therapy (HAART). **Materials and Methods:** A cross-sectional and prospective study was carried out between July 2013 and May 2017 at the antiretroviral therapy centre attached to the Rural Medical College (RMC), Loni, Maharashtra, India. A total of 584 HIV seropositive women, who were clinically diagnosed as suffering from vaginitis were included in the study. Among them, 18 patients lost their lives and were excluded from the study. High vaginal swabs, epithelial scrapings, and vaginal discharge were collected from all the subjects. All samples were processed for microscopy (wet mount, KOH mount, and grams staining), Amsel's score, Nugent's criteria, culture, and antibiotic susceptibility testing using standard laboratory methods. Statistical analysis was done using Microsoft word, and Excel. **Results:** Among the 566 patients followed up for a year, the incidence of vaginitis was observed predominantly in the age group of 24-35 years. 46% of the patients had bacterial vaginosis, and 22.6% suffered from vaginal candidiasis. The occurrence of vaginitis was comparatively more before initiation of antiretroviral therapy (61.1%), than after antiretroviral therapy (38.8%). Occurrence of bacterial vaginitis (58.8%) was noted to reduce after the initiation of antiretroviral therapy (42.5%). Vaginal candidiasis (63.2%) was observed to be significantly reduced after the initiation of HAART (36.7%). Microscopic examination of the vaginal discharge using Amsel's criteria revealed the presence of vaginitis before 49 (31%) and after initiation of HAART 26 (23%) and the Nugent score showed that 84 (53%), and 61 (55%) had signs of vaginitis before and after initiation of HAART respectively. The culture results revealed 265 bacterial and 128 fungal isolates. Among the 265 bacterial isolates 156 (58.8%) were isolated before starting HAART and 109 (41.1%) were recovered after initiation of ART. *Staphylococcus* predominated the bacterial cause of vaginitis, and *Candida albicans* was the most frequent cause of vaginitis both before and after initiation of HAART. 16 (2.8%) patients showed the presence of MRSA. **Conclusion:** This study clearly demonstrates that there is an increased prevalence of vaginitis among the HIV infected population, both before and after initiation of HAART. Regular screening for the cause of vaginitis, initiation of appropriate antimicrobial therapy could contribute to better quality of life among HIV infected patients.

Keywords: HIV/AIDS; Opportunistic infections (OI's) in HIV/AIDS; Vaginitis; HIV; Highly active antiretroviral therapy (HAART); MRSA; *Staphylococcus*; *Candida albicans*; Bacterial vaginosis; Vaginal candidiasis

Introduction

Human immunodeficiency virus (HIV), the causative agent of Acquired immunodeficiency syndrome (AIDS) is a widespread public health problem prevalent throughout the world. Vaginitis is a clinical condition which is associated with social stigma and the lack of understanding it is wreaking havoc on women's health. Genital tract infections are a serious problem when associated with HIV/AIDS, and depleting immunity could

increase the probability of HIV infected women to suffer from vaginitis. Imbalance in microbial colonization is a potential risk

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factor in HIV seropositive women, which could be a cause for subsequent infections later. There are only fewer reports on the prevalence of vaginitis among the general population, and HIV infected women.^[1,2] Although most cases of vaginitis don't require hospitalization and can be treated effectively, the clinical course and the impact of the disease could be contrasting among HIV seropositive women and normal healthy females who are HIV seronegative.

Since HIV infection results in AIDS, presence of vaginitis has remained a major threat in HIV/AIDS disease progression. The incidence of vaginitis was noted to increase among immunocompromised patients. Previous studies have reported that the majority of vaginitis cases were caused by *Candida* spp, *Gardenerella vaginalis*, and *Trichomonas vaginalis*, which are responsible for 10 million official hospital visits in a year.^[3-7] The episodes of vaginitis was found to increase to as high as 72% among HIV seropositive women, with at least one diagnosed episode requiring treatment, and a self-reported case in a year.^[8]

The dynamics of vaginitis among HIV/AIDS patients could be influenced with the age, TCD4+ cell counts, resistance to azoles and other antimicrobial agents, and the emergence of pathogenic microbial species (non-albicans *Candida* group and others), which further worsen the condition, and complicate the healing process.^[9] Therefore, identifying the host factors and treating vaginitis, which could be symptomatic or asymptomatic, and mild or severe assumes greater significance among HIV/AIDS patients.^[10] The center for disease control and prevention (CDC) has recently considered vaginitis as one of the defining condition of AIDS. Vaginal candidiasis, when caused frequently, could be considered as a sign of immunosuppression due to AIDS, and hence it can be referred to as the "disease of the diseased".^[11] High prevalence, increased relapses, and associated complications in HIV disease can make vaginitis as an important, and a relevant clinical problem which needs to be diagnosed and treated appropriately.^[12] The present is aimed to identify the etiology of symptomatic vaginitis among HIV seropositive patients both before and after initiation of HAART.

Materials and Methods

This is a cross-sectional, and a prospective study done between July 2013 and May 2017 at the antiretroviral therapy center attached to the Rural Medical College (RMC), Loni, Maharashtra, India. An informed and written consent was obtained from all the subjects included in the study. Institutional ethical clearance was obtained for the conduction of the research. A total of 584 HIV seropositive women were included in the study and 566 (18 subjects expired) patients were followed up for a year. Depending on the clinical manifestations two sets of samples were collected, which included high vaginal swabs, epithelial scrapings, and vaginal discharge. Care was taken while collecting the samples, which included a thorough cleaning of the adjacent skin before collecting the sample, and only a pure growth was considered as significant. Samples were processed for microscopy (wet mount, KOH preparation (the whiff test), grams stain), and culture (bacteriological and fungal culture). Culture media used included blood agar, MacConkey's agar, Brain heart infusion agar, and Saborauds dextrose agar. Amsel's

clinical criteria (Vaginal P^H, type of vaginal discharge, presence of clue cells, and the odor), and Nugent's score (bacterial counts in grams's stain per oil immersion field) was used to diagnose bacterial vaginosis.^[13,14] The bacterial antibiotic susceptibility testing was performed by Kirby-Bauer disc diffusion method. *Candida* spp were identified and speciated by growing on Hi-chrome agar, sugar assimilation and fermentation tests. An antifungal activity of various drugs against *Candida* was tested using Muller Hinton agar supplemented with 2% glucose and 0.5 µg/ml methylene blue. Methicillin resistant *Staphylococcus aureus* (MRSA) was detected by using cefoxitin 30 µg, and disk diffusion method. CLSI guidelines were used to perform antimicrobial susceptibility testing.^[15-17]

Results

Of the total 566 HIV seropositive women who were clinically suspected to be suffering from vaginitis included in the study, were grouped as 18-24 years, 25-34 years, 35-44 years, and ≥ 45 years. Bacterial vaginitis was observed in 46% patients, and 22.6% suffered from vaginal candidiasis. The occurrence of vaginitis was comparatively more before initiation of antiretroviral therapy (61.1%), than after antiretroviral therapy (38.8%). Occurrence of bacterial vaginitis (58.8%) was noted to reduce after the initiation of antiretroviral therapy (42.5%). Vaginal candidiasis (63.2%) was observed to be significantly reduced after the initiation of HAART (36.7%). Positive culture yielded from 346 (61.1%), and 220 (38.8%) patients before and after initiation of antiretroviral therapy respectively, as shown in Table 1.

Table 1: Positive vaginal cultures in different age groups before and after initiation of HAART.

Age group (n=566)	Before HAART (n=346)	After HAART (n=220)	p-value
18-24 years	54	33	0.983
25-34 years	147	97	0.983
35-44 years	69	42	0.983
≥ 45 years	76	48	0.983

265 bacterial species were isolated and 128 *Candida* spp yielded from the cultures. Among the bacterial isolates in patients screened before initiation of HAART, *Staphylococcus* spp (36.5%) predominated followed by *Escherichia coli* (22.4%), *Klebsiella* spp (18.5%), *Pseudomonas* spp (13.4%), *Proteus* spp (7%), *Neisseia gonorrhoeae* (3.2%), and *Gardnerella vaginalis* (1.9%). After initiation of HAART there was a comparative reduction in the isolation of bacteria as shown in Table 2.

Table 2: Bacterial isolates before and after initiation of antiretroviral therapy.

Bacterial species	Before HAART (n=156)	After HAART (n=109)	p-value
<i>Staphylococci</i>	57	36	0.193
MRSA	16	3	0.193
<i>E. coli</i>	35	25	0.193
<i>Klebsiella</i>	29	11	0.193
<i>Pseudomonas</i>	21	7	0.193
<i>Proteus</i>	11	5	0.193
<i>Gardenella</i>	3	1	0.193
<i>Nissieria gonococci</i>	5	-----	0.193

MRSA: Methicillin resistant *Staphylococcus aureus*

Microscopy and evaluation of the specimens collected before initiation of HAART by the Amsel's criteria and the Nugent's score showed positivity in 49 (31%), and 84 (53%) respectively. Post-HAART, the signs of vaginitis were noted to be 26 (23%), and 61 (55%) using Amsel's criteria and Nugent's score respectively as shown in Table 3.

Table 3: Evaluation of bacterial vaginitis using Amsel's and Nugent's criteria before and after initiation of HAART.

Criteria	Before HAART (n=156)	After HAART (n=109)	p-value
Amsel's criteria	49 (31%)	26 (23%)	0.2886
Nugent's score	84 (53%)	61 (55%)	0.2886

Gram's stain revealed the presence of *Trichomonas vaginalis* (2.8%) among seven patients who were HAART naive. Fungal culture of the specimens before HAART yielded *Candida albicans* (33.5%), followed by *C tropicalis* (16.4%), *C krusie* (7%), *C glabarata* (5.4%), and *C dublinensis* (0.7%) as shown in Table 4.

Table 4: Fungal culture results before and after antiretroviral therapy.

Organism	Before HAART	After HAART	p-value
<i>C. albicans</i>	43	21	0.0082*
<i>C. glaberta</i>	7	15	0.0082*
<i>C. tropicalis</i>	21	10	0.0082*
<i>C. krusie</i>	9	1	0.0082*
<i>C. dublinensis</i>	1	-----	0.0082*

*Significant

Discussion

Anatomically, inflammation/infection of the vulva, its ectodermal origin structures, and cervix with external and internal orifice is termed as vaginitis.^[18] Vaginitis/vulvovaginitis was first described by Wilkinson in 1849.^[19] When bacteria other than *Gardnerella vaginalis* are responsible for the infection/inflammation of vagina, it is termed as "bacterial vaginosis" (BV).^[20] It is referred to as *Gardnerella vaginitis* (when vaginitis is caused by *Gardnerella vaginalis*), and vulvovaginal candidiasis when caused by *Candida* spp. *Candida* is a much adaptable opportunistic pathogen causing a variety of infections, and its high adaptability in HIV infected patients could complicate the patient management. Though *Candida* is a normal commensal among healthy individuals, its role in causing vaginitis in HIV/AIDS patients assumes greater significance. The etiology of BV is not clearly understood, despite its high prevalence both among HIV seropositive patients and general population. A previous study has noted that there is a 50-60% increased risk of vaginitis among individuals with high-risk sexual behavior.^[21] HIV/AIDS results in immunosuppression, and people infected with HIV are prone to opportunistic infections, which could be responsible for the severe morbidity and mortality. Especially, vaginitis in HIV/AIDS patients, either symptomatic/mildly-symptomatic/asymptomatic, needs to be evaluated, which in turn could provide a treatment guide for clinical microbiologists and clinicians to effectively manage HIV/AIDS patients.^[9]

It should be noted that the characteristics of the microorganisms causing infections in normally healthy individuals might differ

from those which cause infections in HIV/AIDS patients. The microbes which are less/non-virulent and non-pathogenic usually do not cause infections in individuals with intact immune system, but can be responsible for fatal infections in HIV/AIDS patients. Clinical course of vaginitis among infected population may vary extremely (localized to systemic involvement), depending on the nature of microorganism involved, and the immunological status of the individual. Episodes of vaginitis are common among women of various age groups and are usually self-limiting (not needing hospitalization and antimicrobial therapy). HIV/AIDS patients suffer from recurrent episodes of vaginitis and may require hospital visits and prolonged antibiotic treatment.^[9]

In the present study bacterial vaginosis was predominant followed by vulvovaginal candidiasis before starting HAART. Vaginitis was noted to be more predominant and persistent among HIV seropositive women before and after initiation of HAART as noted by the Nugent's criteria. There was a high prevalence of vaginitis in the 24-35 year age group. Isolation of *Staphylococcus* species among the symptomatic patients signifies their importance as potential pathogens responsible for vaginitis among HIV seropositive patients. Vaginal candidiasis showed a considerable reduction after initiation of antiretroviral therapy. Nugent's criteria could diagnose more cases of vaginitis as compared to the Amsel's score.

Most bacterial isolates were found to be sensitive to imipenem, piperacillin/tazobactam, Amoxicillin-clavulanic acid, amikacin, ceftriaxone, ofloxacin, cefotaxime, cefaperazone, and ceftazidime. Decreased sensitivity was observed against co-trimoxazole, and gentamicin. All *Staphylococcus* isolates showed susceptibility to vancomycin and linezolid. Commonly used antifungal agents (amphotericin B, fluconazole, clotrimazole, and nystatin) were tested against *Candida* isolates. Most *Candida albicans* isolates were found to be susceptible to amphotericin B, fluconazole, clotrimazole, and nystatin. A low level of drug resistance was observed among non-albican's *Candida* species. Methicillin resistant *Staphylococcus aureus* was observed in 2% cases of BV.

This is a first of its kind study which evaluated the etiology of vaginitis among HIV seropositive patients and compared the differences in the causative agents both before and after initiation of antiretroviral therapy.

Incidences of vaginitis in the general population, as reported by a previous study from North India by Bhalla et al. had noted that there was a 32.8% prevalence of bacterial vaginosis in Delhi, and revealed that 31.2% patients were asymptomatic. The same study had observed that infection with *Trichomonas vaginalis* could be a predisposing factor for chronic bacterial vaginitis.^[22] Another report by Madhivanan et al, from South India showed a prevalence of 19% among young and sexually active women.^[23] A study of genital tract infections by Mohapatra et al, from Orissa (East of India), revealed a staggering 66% prevalence of bacterial vaginitis.^[24] A study from Mumbai (India), by Narayankhedkar et al. showed a prevalence rate of 28.9% among women of reproductive age.^[25]

There are only few studies available in literature, which have evaluated the prevalence of vaginitis among HIV seropositive patients. A recent report by Goel et al. from North India has observed 30% prevalence of bacterial vaginosis among HIV seropositive women. The same study also revealed *Candida* sp. (10%), and mixed infections (30%) as other agents responsible for vaginitis.^[26] A recent report by Lulla et al. from India studied the prevalence of lower genital tract infections among HIV seropositive women, both before and after initiation of ART. The results of this study had showed the prevalence of bacterial vaginosis (47.7%), vaginal candidiasis (43.2%), and trichomoniasis (8.8%). This study has also observed that there was a positive correlation of vaginitis with the TCD4+ cell counts.^[27] Another report by Bhattar et al, who correlated the TCD4+ cell counts, plasma HIV/RNA viral load, and lower genital tract infections, found a 9% prevalence of bacterial vaginitis and no statistical significance of TCD4+ cell counts with the etiology of vaginitis.^[28]

As evidenced from the available literature, the prevalence of vaginitis appears to vary with the geographical region, age of the individual, their immunological status, and other factors. This study signifies the importance of *Staphylococcus*, MRSA, *E coli*, and *Klebsiella* spp., like other pathogens causing BV with high potential to develop antibiotic resistance. BV and vaginal candidiasis has a high recurrence rate, which causes difficulty in treating the condition. Therefore, identification of the causative microbe and antimicrobial susceptibility testing is necessary.

Antimicrobial chemotherapy, to treat vaginitis among HIV infected population requires prolonged treatment, which could restore the vaginal flora (Lactobacilli dominated environment).^[29,30]

Conclusion

As evidenced from the study results, and the available literature, the prevalence of vaginitis appears to vary with the geographical region, age of the individual, their immunological status, and other factors. HIV seropositive patients suffer from several opportunistic infectious conditions, which account for the severe morbidity and mortality. Vaginitis among HIV infected patients could result in increased morbidity, and decrease the quality of life. Inclusion of microbiological screening for evaluating the etiology, and antimicrobial susceptibility testing could act as a guide to better management of HIV infected women, both before and after initiation of antiretroviral therapy.

Conflict of Interest

All authors disclose that there was no conflict of interest.

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