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Research Article

Genital Infections of HIV-Infected Women Assisted by a Specialized Service

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The present study aimed at analyzing the persistence/recurrence of genital infections and its associated factors in HIV-infected women. Fifty-eight women treated for chlamydial infection, trichomoniasis, vulvovaginal candidiasis, and/or bacterial vaginosis (BV) and who had specimens collected for cure control up to one year after treatment were studied. Diagnoses were performed by the Gram staining method for cases of BV and candidiasis and by *T. vaginalis* culture and qualitative PCR for *C. trachomatis*. Antiretroviral therapy was used by 79.3% of patients, and 62.1% showed an undetectable HIV plasma load. The most frequent infection was BV with persistence/recurrence of 52.4%, which was associated with a longer time period between treatment and cure control ($P = 0.0455$), postmenopausal period ($P = 0.0451$), and having a steady partner ($P = 0.007$). Persistence/recurrence of vulvovaginal candidiasis was observed in 25%, trichomoniasis in 23.1%, and chlamydial infection in 10.5%. The latter was associated with inadequate treatment of the partner ($P = 0.02$). There was a tendency to higher persistence/recurrence of BV ($P = 0.063$) and trichomoniasis ($P = 0.073$) among patients with low T $CD4^+$ lymphocyte counts. The majority of women in the present study showed good HIV-infection control and a vulnerable sexual behavior, which stress the importance of maintaining gynecological followup.

1. Introduction

With the increase of HIV infection among adolescent and young adult women, gynecological manifestations play an important role in its transmission [1–3].

Sexually transmitted diseases (STD) and other infections in the genital tract are related to increased susceptibility to HIV infection and to a higher potential risk of transmission [2–9]. Due to synergy among etiological agents, viral RNA levels in the lower genital tract are increased, which favors transmission [2, 7–9].

HIV infection, on the other hand, can increase the incidence, persistence, and recurrence of genital infections, such as bacterial vaginosis (BV) [10–12], trichomoniasis [13, 14] and vulvovaginal candidiasis [15, 16]. Ghosh et al. [17] showed higher prevalence of chlamydial infection in HIV-infected women with low levels of T $CD4^+$ cells; however, little information can be found on *Chlamydia trachomatis* infection in these patients.

The use of antiretroviral drugs, the T $CD4^+$ cell counts, and the HIV viral load seem to influence the rates of incidence, persistence, and/or recurrence of BV [10–12] and candidiasis [15, 16, 18–20]. HIV-infected women show higher risk for vaginal colonization and infection by *Candida* species, which is reduced by antiretroviral therapy (ART) [20, 21]. In the case of vaginal trichomoniasis, recurrence seems to be more related to sexual behavior than to therapeutic failure [14, 22].

Trichomoniasis favors the occurrence of BV [23], and coinfection with the two microorganisms may hinder the treatment of both diseases in HIV-infected patients [24]. BV causes cellular alterations in the cervix [25], and similarly to trichomoniasis, favors the acquisition of human papillomavirus (HPV) [26]. Additionally, trichomoniasis and *C.*

trachomatis infection are associated with inflammatory pelvic disease in HIV-infected women [27, 28]. Hence, it is of utmost importance to control such genital infections in these patients.

In addition, a high percentage of women are asymptomatic to some of these infections, making the active search for such infections and the cure control important items to be pursued [13, 14, 17, 22].

The present study aimed at analyzing the persistence and/or recurrence of chlamydial infection, trichomoniasis, candidiasis and BV in HIV-infected women and at relating such rates to variables viral load, T $CD4^+$ count and sexual behavior.

2. Patients and Methods

A descriptive and analytical study was conducted in the Service of Specialized Infectology Outpatient Units "Domingos Alves Meira," at the Botucatu School of Medicine Complex-UNESP. This is a reference service that assists HIV-infected/AIDS patients in the micro-region of Botucatu (SP), under the jurisdiction of the Bauru Regional Health Department-DRS VI-Bauru (SP).

One hundred and eighty-four women infected with HIV/AIDS were studied from 2008 to 2012. Of these, 120 showed a diagnosis of bacterial vaginosis, vulvovaginal candidiasis, and infections by *Trichomonas vaginalis* and/or *Chlamydia trachomatis*. Of the infected group, 58 women were enrolled in this study, which was conducted from 2011 to 2012, according to the inclusion criteria.

The inclusion criteria were as follows: having a confirmed diagnosis of HIV infection, being 18 years old or older, having initiated sexual activity, showing physical and mental conditions to participate in the study, not being pregnant, having been treated specifically for the abovementioned infections, and having returned for a new examination up to one year after the first treatment, in addition to having agreed to participate in the investigation by signing a free-consent form.

The patients who tested positive for BV, vulvovaginal candidiasis, trichomoniasis and/or infection with *C. trachomatis,* as well as sexual partners of those with trichomoniasis, and infection by *C. trachomatis,* were treated according to recommendations from the 2005 Manual for Control of Sexually Transmitted Diseases (STD) of the Brazilian Ministry of Health. The patients were called for cure control collections between 40 and 60 days after treatment, as recommended [29]. However, new collections were performed from all patients reporting to the service after the treatment for a period of up to one year.

The control tests were performed following the same diagnostic methods. Gynecological examination and material collection were performed by one of the authors for evaluation of these genital infections. The vaginal content and cervical secretion samples were analyzed by the Department of Pathology of the Botucatu School of Medicine, UNESP.

Vulvovaginal candidiasis was diagnosed by the visualization of blastoconidia and/or pseudohyphae under microscopic examination of vaginal smear stained by the Gram method. BV diagnosis was performed by considering the criteria established by Nugent et al. [30], based on the morphology and number of microorganisms observed in the vaginal smear stained by the Gram method. The study on protozoan *T. vaginalis* was performed by culture after seeding the vaginal content in Diamond's liquid medium, followed by incubation at a temperature of 37° for 72 hours, and reading by a light microscope. The search for *C. trachomatis* in secretion was performed by qualitative polymerase chain reaction (PCR). For this purpose, DNA was extracted by using CTAB solution, amplifying nucleic acid, and visualizing the amplified products in agarose gel.

Positive results for the abovementioned infections after the treatment were considered as persistence or recurrence.

Sociodemographic, behavioral, and clinical data were obtained by means of structured interviews.

T $CD4^+$ lymphocyte count and HIV viral load determination were performed in the routine of the care provision service to HIV/AIDS patients by the Botucatu Blood Center-UNESP.

Frequency and percentage were used for qualitative variables and means and standard deviations for quantitative variables. The associations between qualitative variables were performed by the chi-square test or Fisher's exact test, whenever necessary. The quantitative variables were compared, in relation to recurrence or not, by Student's t-test. A level of significance of 5% of probability or its corresponding P value was used for all tests.

The research project was approved by the Research Ethics Committee of the Botucatu School of Medicine in December 2010.

3. Results

Fifty-eight HIV-infected women who had been diagnosed with and treated for the genital infections studied were enrolled according to the inclusion criteria established. Most of them reported to be white (70.7%), in the menacme period (77.6%), undergoing ART (79.3%), and had undetectable HIV plasma load (62.1%) (Table 1).

BV was the most prevalent isolated infection among these women (36.2%) or in association with chlamydial infection (17.2%). Twenty-three (39.6%) patients showed association of genital infections (Table 2).

Of the 58 women studied, 42 received treatment for BV, including those with association of infections. Twenty-two (52.4%) patients showed persistence/recurrence of the infection. The factors associated with BV persistence/recurrence were higher mean age ($P = 0.017$), being menopausal ($P = 0.0451$), longer time between treatment and cure control ($P = 0.0455$), and having a steady partner ($P = 0.007$). Higher BV persistence/occurrence was observed in patients with T $CD4^+$ lymphocyte count lower than 200 cells/mm^3 ($P = 0.063$). No difference concerning the use of condoms ($P = 0.193$), ART ($P = 0.120$), and viral load quantification ($P = 0.801$) was observed (Table 3).

Eight patients, out of the 58 enrolled in the study, had been treated for vulvovaginal candidiasis, and two of them

Table 1: Characterization of 58 HIV-positive women with genital infection.

Variables	Mean or N	Standard deviation or %
Age	41.55	9.6
Skin color		
White	41	70.7
Non-white	17	29.3
Years of schooling concluded		
0 to 3 years	10	17.2
4 to 7 years	23	39.6
8 to 11 years	17	29.3
≥12 years	08	13.8
Phase of reproductive life		
Menacme	45	77.6
Postmenopause	13	22.4
Use of hormonal contraceptives		
Yes	06	10.4
No	52	89.7
Partners in the past year		
None	13	22.4
A steady partner	34	58.6
Two or more	13	22.4
Use of condoms (N = 45*)		
Consistent use	26	57.8
Inconsistent use	19	42.2
Use of alcohol		
Yes	22	37.9
No	36	62.1
Use of tobacco		
Yes	20	34.5
No	38	65.5
Use of antiretroviral therapy		
Yes	46	79.3
No	12	20.7
T CD4+ Lymphocyte Count/mm^3		
<200	08	13.8
200 to 500	24	41.4
>500	26	44.8
HIV viral load (copies/mL)		
Undetectable	36	62.1
Detectable	22	37.9
Total	58	100

*13 patients without sexual activity were excluded from this item.

(25.0%) showed persistence/recurrence of infection in a one-year period. Four patients were diagnosed with associated candidiasis and BV (50.0%). Vulvovaginal candidiasis persistence/recurrence could not be associated with any risk factors.

Table 2: Distribution of 58 HIV-positive patients according to the genital infection diagnosed.

Infection diagnosed	N (%)
Bacterial vaginosis (BV)	21 (36.2)
Vulvovaginal candidiasis (VC)	03 (05.2)
Chlamydia trachomatis (CT)	06 (10.4)
Trichomonas vaginalis (TV)	05 (08.6)
BV + VC	03 (05.2)
BV + CT	10 (17.2)
BV + TV	07 (12.1)
VC + CT	01 (01.7)
TV + CT	01 (01.7)
BV + VC + CT	01 (01.7)
Total	58 (100)

Thirteen of the 58 patients of the study had been treated for *T. vaginalis* infection, and three (23.1%) of them showed persistence/recurrence of infection. All patients with *T. vaginalis* reported active sexual life. Persistence/recurrence of infection was not significantly related either to the number of partners (P = 0.503) or to condom use (P = 0.202). Higher tendency to persistence/recurrence was observed among the patients with lower TCD4$^+$ levels (P = 0.073) and smokers (P = 0.069), and there was no difference in relation to viral load (P = 0.510).

Among the 13 patients treated for *T. vaginalis,* seven (53.8%) were also diagnosed with BV, and two of these (28.6%) showed persistence/recurrence of both infections, and another two (28.6%) of BV only. The other three (42.9%) patients showed cure for both infections.

Two (10.5%) of the nineteen patients that had been treated for *C. trachomatis* showed persistence/recurrence of the infection. These two patients reported partners who have not been properly treated, and this was the only occurrence associated with persistence/recurrence of the chlamydial infection (P = 0.0278).

4. Discussion

According to their sociodemographic aspects, the women included in the present study were characterized by living in São Paulo state. They were predominantly white, in the menacme period and with poor schooling. Such characteristics are similar to those found in the study of Duarte [31] on a larger series of patients from the same service.

In the present study, the proportion of white women (70.7%) was higher than what is reported to women with HIV/AIDS in São Paulo state [32], in Brazil [33], and in North American cohorts [3, 22]. The proportion of women with poor schooling was similar to that reported in São Paulo state and in Brazil [32, 33]. However, the proportion of women with 12 or more years of schooling was larger in the present group of subjects.

Regarding sexual behavior, similar vulnerability to HIV infection and to other sexually transmitted diseases was found between this study and that performed by Duarte [31].

Table 3: Distribution of 42 HIV-infected women and genital infection, according to factors associated with bacterial vaginosis (BV) persistence/recurrence.

Variables	BV− Mean or N (standard deviation or %)	BV+ Mean or N (standard deviation or %)	P value
Age	36.65 (9.59)	45.59 (7.55)	0.017
Phase of reproductive life			
Postmenopause	02 (20.0)	08 (80.0)	0.0451
Menacme	18 (56.2)	14 (43.8)	
Time of collection after treatment			
<60 days	10 (66.7)	05 (33.3)	0.0455
60 days to 06 months	06 (37.5)	10 (62.5)	
06 months to 01 year	04 (36.4)	07 (63.6)	
Number of partners in the past year			
None	03 (30.0)	07 (70.0)	0.007
One	10 (40.0)	15 (60.0)	
02 or more	07 (100)	—	
Use of condoms (N = 32)*			
Yes	09 (52.9)	08 (47.1)	0.193
No	07 (46.7)	08 (53.3)	
Use of antiretroviral therapy			
Yes	17 (51.5)	16 (48.5)	0.120
No	03 (33.3)	06 (66.7)	
T $CD4^+$ cell count			
<200 cells/mm^3	03 (37.5)	05 (62.5)	0.063
Between 200 and 500 cells/mm^3	07 (41.2)	10 (58.8)	
>500 cells/mm^3	10 (58.8)	07 (41.2)	
Viral load			
Undetectable	12 (46.2)	14 (53.8)	0.801
750 copies	08 (50.0)	08 (50.0)	
Total	20 (47.6)	22 (53.4)	

*10 patients without sexual activity in the past year were excluded from this item.

And although most participants (58.6%) reported having only one sexual partner in the past year, the proportion of women with multiple partners in this study (22.4%) was higher than in the Brazilian population [34]. Additionally, only 57.8% of the women in this study reported consistent use of condoms. This proportion is higher than in the general Brazilian population [35] but lower than what is reported by HIV/AIDS reference centers in Brazil [36] and in other countries [2, 3, 37].

The high percentage of ART use (79.3%) and the appropriate clinical control of HIV infection, as measured by the undetectable HIV plasma viral load, in 62.1% of women and by the T $CD4^+$ cell count above 500 cells/mm^3, in 44.8%, denote the quality of the medical care provided. These results also differentiate positively in the subjects in this study from most of the HIV-infected populations studied by other authors [2, 3, 9–16, 22].

The genital infection most frequently found among the patients in this study was BV, which agrees with other investigations [2, 3, 10, 11] showing high BV prevalence in HIV-infected patients. In addition to prevalence, Jamieson et al. [10] observed higher BV persistence and severity in women with HIV, which might be related to the greater presence of fastidious bacteria in their vaginal microbiota, according to the results of Mitchell et al. [38].

In the present study, 52.0% of BV was diagnosed after treatment, thus characterizing persistence/recurrence. Persistence could not be distinguished from recurrence because patients would not return for cure control at the specified moments of the followup. It was also observed that the longer the time between treatment and control, the lower the percentage of cure (P = 0.045), which could predict greater chances of BV recurrence over time.

BV recurrence in the population that is not HIV-infected ranges from 19.9% to 58.0% [39–41]. This may be related either to the lack of eradication of the microorganism [42] or to behavioral factors that enable reinfection [39, 40, 43, 44]. Although there are few studies on BV recurrence in HIV-infected women, some authors [41, 45] relate the greater recurrence of the microorganism to the lack of condom use by this population. However, such relation was not observed in this study.

According to some researchers [43, 44, 46], the higher recurrence rate among patients who use condoms irregularly and have multiple partners reinforces the hypothesis of sexual

BV transmission. However, Bradshaw et al. [40] observed a higher recurrence of the infection among non HIV-infected women with a steady partner, as it was observed in the present study on HIV-infected women. According to those authors [40], the data suggest that they are reinfected by their steady partners. Nevertheless, other studies [47, 48] did not find decreased recurrence after treatment of the partners. Hence, there is no agreement regarding the influence of sexual practices on BV recurrence.

Some studies [10–12] showed increased BV occurrence with low T $CD4^+$ counts, suggesting that immunodeficiency favors the unbalance of the vaginal flora and BV occurrence. Nevertheless, there are no known studies which effectively associate BV recurrence with $CD4^+$ levels. In the present study, there was only a tendency to higher BV persistence/recurrence in patients with lower $CD4^+$ levels ($P = 0.063$).

The rate of hormonal contraceptive use, considered to be a protective factor against BV [39, 40, 44], was low (10.4%) in the studied sample; however, it was not related to higher persistence/recurrence, probably due to the small number of patients involved. Nevertheless, there was an association between BV persistence/recurrence and postmenopause ($P = 0.04$), and none of the patients had hormonal replacement. Some studies [49, 50] show greater BV occurrence in postmenopause, which decreases with hormone replacement therapy, reinforcing the hypothesis of the hormonal role in the protection against BV. However, BV diagnosis by Nugent's criteria is not well established in menopausal women [51], which could hinders analysis.

Vulvovaginal candidiasis was infrequent among the patients in this study. Although some authors [15, 16, 18–21] have shown higher prevalence of that infection in HIV-infected patients through a culture of the vaginal secretion for *Candida* species, others [2, 3] found lower prevalence using Gram staining, a method with better diagnostic accuracy [52].

The small number of women with vulvovaginal candidiasis included in this study (eight) did not allow for relating persistence/recurrence factors. Some studies [16, 18, 20] report high rates for vulvovaginal candidiasis persistence and recurrence in HIV-infected patients, particularly in those with low T $CD4^+$ counts and high viral loads [18, 20]. The majority of the women in this study were using ART (79.3%), which is considered to be a protective factor against candidiasis in general [20, 21]. They also showed an undetectable viral load (62.1%) and T $CD4^+$ counts higher than 200 cells/mm^3 (86.2%), which could explain the low frequency of vulvovaginal candidiasis found. Additionally, the use of contraceptive pills, considered to be a possible triggering factor for the infection [53], was low in the studied population. Nevertheless, it was not possible to evaluate its influence on the persistence/recurrence of the infection.

In the preset study, the proportion of trichomoniasis persistence/recurrence was 23%. This is in agreement with the rates reported by other authors [13, 14, 54] for HIV-infected patients (18–36%), which are higher than for the noninfected population (5%–8%) [55, 56]. Recurrence may be related to treatment failure due to antibiotic resistance, to improper treatment, or to reinfections [14, 22, 54, 57].

There was a tendency to higher persistence/recurrence among patients with lower T $CD4^+$ levels ($P = 0.073$). Several studies [13, 14, 22] did not relate low T $CD4^+$ levels to *T. vaginalis* occurrence and report the reinfection by untreated partners or the acquisition from a new partner as main recurrence factors. Cu-Uvin et al. [22] compared women at high risk for infection with others already infected with HIV and found similar trichomoniasis prevalence and recurrence between the two groups. The authors suggested that other factors, such as sexual behavior, more than the immune status would be related to that infection.

In the present study, persistence/recurrence of the infection was not related to the number of partners or condom use; however, the small number of patients with trichomoniasis included (13) and showing persistence/recurrence (three) hindered analysis.

Gatski and Kissinger [57], however, showed recurrent *T. vaginalis* in patients without sexual reexposure, thus indicating a probable treatment failure with the agent persisting and later recurring, unrelated to sexual practices. Hence, treatment failure may be an important cause of recurrence. Although several studies indicate a single dose of oral metronidazole as the first alternative for treatment, Kissinger et al. [58], in a randomized clinical study on HIV-infected women, showed that oral metronidazole for seven days was more effective in eradicating *T. vaginalis*.

Factors as being black and smoking are related to higher *T. vaginalis* recurrence in HIV-infected patients [13, 14, 22]. In the present study, there was a tendency to higher *T. vaginalis* persistence/recurrence in smokers ($P = 0.069$). And as the prevalence of black women in the studied population was low, this factor could not be related to higher recurrence.

The association between BV and trichomoniasis is frequent in HIV-infected patients, and it may bring difficulties to the treatment of both diseases [23, 24]. In the present study, 53.8% of patients with trichomoniasis also showed BV, with 28.6% of recurrence for both infections, which is in agreement with those authors.

Some authors [1, 13, 14] consider trichomoniasis the most frequent STD among HIV-infected women; however, in the present group of patients infection with *C. trachomatis* was more frequent. This finding was coherent with that observed by Duarte [31] in a population assisted by the same service, with high *C. trachomatis* prevalence (24.6%) as compared to other studies [1, 17, 59, 60].

Although some studies [17, 60] report higher prevalence of chlamydial infection in HIV-infected patients, no data were found showing the proportion of recurrence of such infection in HIV-infected women. In the present study, there was 10% persistence/recurrence of *C. trachomatis* infection related to inappropriate treatment of the patients' partners ($P = 0.02$), a rate that was within the variation found (5%–29%) in different populations not infected with HIV [61–63]. Most of these cases are related to reinfection by partners that were inappropriately treated or by new partners [61–63]. Schillinger et al. [64] showed reduced recurrence

when partners are treated, suggesting the importance of reinfection by sexual contact.

In the present study, no associations were observed between persistence/recurrence of chlamydial infection and sexual behavior, T $CD4^+$ lymphocyte levels, or HIV viral load.

One limitation of this study that hindered data analysis was the small number of subjects, since patients frequently escaped from followup after treatment. This investigation points out the importance of adherence to medical followup and cure controls, particularly in the case of infections that usually exhibit high rates of persistence and recurrence.

5. Conclusion

The majority of women in the present study showed good control of the HIV infection, with low plasma viral loads, ART use, and T $CD4^+$ levels above 200 cells/mm^3. However, vulnerable sexual behavior was observed, with a high percentage of women with multiple partners and inconsistent condom use.

BV was the most frequent genital infection. It also showed a high persistence/recurrence rate (52.4%), thus indicating the need for adherence to cure controls and for searching more effective treatments. Vulvovaginal candidiasis was infrequent, which may be due to efficient HIV-infection control or to the diagnostic method used. The persistence/recurrence of this infection was 25.0%; however, it was not possible to relate it to any variables due to the small size of the sample. Infection with *C. trachomatis* occurred more frequently than with *T. vaginalis*, although with a persistence/recurrence rate (10.5%) lower than in trichomoniasis (23.1%).

There was a tendency to higher persistence/recurrence of BV and trichomoniasis in patients with lower T $CD4^+$ levels, which may indicate the influence of their immunologic status. However, no association was found between the HIV viral load and persistence/recurrence of genital infections.

Having a single partner was related to higher BV persistence/recurrence, but sexual behavior was not associated to trichomoniasis. As to infection with *C. trachomatis*, the treatment of sexual partners was of utmost importance for the patients' cure and reduction of recurrence rates.

The highest BV persistence/recurrence was also related to the longer time between treatment and control collection and to being postmenopausal. These findings show the importance of regular gynecological followup and the likelihood of association of BV persistence/recurrence with hormonal factors.

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