

Prevalence of vaginal infection by candida subtypes in women with HIV compared to the control group and influence of immune status

Prevalência da infecção vaginal pelos subtipos da cândida em mulheres com HIV comparativamente ao grupo controle e influência do estado imunológico

Somaia Reda^{1,2} , Ana Cecília Pedriali Guimarães Spautz¹, Isabela Gil², Maria Victoria Fujii Kato², Elizabeth Souza Frade Coltro³, Mariana Hiromi Azuma³, Viviane Obialski¹ , Newton Sérgio de Carvalho¹ 

ABSTRACT

Introduction: Vulvovaginal candidiasis (VVC), caused by *Candida* sp., may present with increased frequency and symptoms in immunocompromised patients, and there is concern about the diagnosis and adequate treatment of these patients. **Objective:** The aim of this study was to evaluate the prevalence of VVC compared to the immune status and species present in the microbiota of patients with HIV. **Methods:** This cross-sectional study was conducted from January 2017 to January 2018, including 97 women with HIV compared to a control group (n=112). For data collection, interviews were conducted, vaginal pH was measured, CD4+ T cells and viral load (VL) were evaluated, and vaginal secretions were collected for microscopy and culture. **Results:** The prevalence of VVC was 41.2% in seropositive patients and 3.6% in the control group (p<0.001). Of the patients with CD4+<200 T, 54.3% had *Candida* sp., while in those with CD4+ T ≥200 cells/mm³, the prevalence was 33.9% (p=0.057). Regarding viral load, the prevalence of VVC was 24.1% in those with undetectable VL or <20, 50% in those with VL between 20 and 9999, and 46.9% in VL above 10000 copies/mL (p=0.08). The most prevalent species were *Candida albicans*, followed by *C. parapsilosis*, *C. krusei*, and *C. glabrata*. The mean vaginal pH was 4.6 in the control group and 5.2 in the case group. **Conclusion:** HIV-positive women have a higher prevalence of candida. Lower T CD4+ values and increased VL seem to be associated with a higher prevalence of infection.

Keywords: *Candida* sp. HIV. Viral load. T CD4+.

RESUMO

Introdução: A candidíase vulvo-vaginal (CVV), causada pela *Candida* sp., pode apresentar-se com aumento da frequência e dos sintomas em pacientes imunodeprimidas, havendo preocupação acerca do diagnóstico e tratamento adequado dessas pacientes. **Objetivo:** Avaliar a prevalência de CVV comparativamente ao estado imunológico e espécies presentes na microbiota de pacientes com HIV. **Métodos:** Estudo transversal, realizado de janeiro de 2017 a janeiro de 2018, incluindo 97 mulheres com HIV comparadas a um grupo controle (N=112). Para a coleta de dados, foram realizadas entrevistas, medida do pH vaginal, avaliação de células T CD4+ e da carga viral (CV) e coleta de secreção vaginal para microscopia e cultura. **Resultados:** A prevalência de CVV foi de 41,2% nas pacientes soropositivas e 3,6% no grupo controle (p<0,001). Das pacientes com T CD4+<200, 54,3% apresentaram *Candida* sp., enquanto naquelas com T CD4+ maior ou igual a 200 células/mm³ a prevalência foi de 33,9% (p=0,057). Com relação à carga viral, a prevalência de CVV foi de 24,1% nas com CV indetectável ou <20, 50% naquelas com CV entre 20 e 9.999 e de 46,9% quando CV acima de 10.000 cópias/mL (p=0,08). As espécies mais prevalentes foram *Candida albicans*, seguida por *C. parapsilosis*, *C. krusei* e *C. glabrata*. A média do pH vaginal foi 4,6 no grupo controle e 5,2 no grupo caso. **Conclusão:** Mulheres positivas para HIV apresentam maior prevalência de cândida. Menores valores de T CD4+ e CV aumentada parecem estar associados a maior prevalência da infecção.

Palavras-chave: *Candida* sp. HIV. Carga viral. T CD4+.

INTRODUCTION

The normal vaginal flora is basically composed of one or two species of *Lactobacillus* sp⁽¹⁾ that make this environment healthy due to its dominance⁽²⁾ and depend on the vaginal pH for its survival. In most women of reproductive age, there is a predominance of the species *L. crispatus*, *L. inners*, and *L. gasseri*⁽³⁾. When there is an alteration in this environment, some microorganisms can overlap the lactobacilli causing diseases such as vulvovaginal candidiasis (VVC) and bacterial vaginosis (BV), and the main problem is that this can occur in apparently healthy women but who have a deficiency or complete absence of these bacteria⁽³⁾.

The vagina is an acidic environment, which can be maintained by both lactobacilli and other microorganisms that act against the proliferation of pathogens due to the production of hydrogen peroxide, toxic hydroxyl radical antibiotics, probiotics, and bacteriocins⁽¹⁻³⁾. The vagina is considered “unhealthy” when it has a great diversity of bacteria but with an increased number of anaerobic bacteria and a decrease in lactobacilli⁽²⁾. For this reason, the production of lactic acid can be essential in maintaining a healthy ecosystem, regardless of the bacterial species that may be present in the vagina.

The composition of the vaginal flora is quite variable in response to exogenous and endogenous factors. These factors include medications such as antibiotics and those with immunosuppressive properties, products used in bathing, use of contraceptives, sexual behavior, pregnancy, and phases of the menstrual cycle. During the menstrual cycle, hormonal variations end up interfering with the metabolism of different microorganisms, and being added to menstrual blood,

¹Universidade Federal do Paraná – Curitiba (PR), Brazil. E-mails: somaia.reda@gmail.com; viviane.obialski@gmail.com; newtonsdsc@gmail.com

²Universidade Positivo – Curitiba (PR), Brazil.

³Hospital de Clínicas da Universidade Federal do Paraná – Curitiba (PR), Brazil.

hormones cause changes in vaginal pH. Other factors that interfere with the composition of the vaginal ecosystem are race and ethnicity, genetic polymorphism, innate or acquired immunity, and the presence of biofilms⁽³⁾.

Vulvovaginal candidiasis is an important disease that causes an imbalance in the vaginal flora, affects 70–75% of women at some point in their lives⁽⁴⁾, and presents risk factors such as sexual activity, pregnancy, diabetes, use of oral contraceptives, and antibiotics, and can occur most often in healthy women. *Candida albicans* is acid-tolerant, and according to the studies by Linhares et al.⁽³⁾, it is found in approximately 10–20% of women of reproductive age. The vaginal pH is acidic and usually ranges from 3.8 to 4.2 due to the presence of *Lactobacillus* sp. When this microbiota is modified by the presence of other bacteria, the pH increases above 4.5, facilitating the contamination of other STIs. In general, the concentration of *Candida* sp is low, making colonized women (carriers) asymptomatic⁽³⁾. However, the group that deserves more attention when diagnosed with candidiasis is HIV-positive, as the incidence, recurrence, and intensity of symptoms are higher in these patients⁽⁵⁾.

Ohmit et al.⁽⁶⁾ showed that oral candidiasis depends more on the CD4 count than on the patient's viral load, and that in the case of vaginal candida it is reverse, which makes us imagine that although the fungus is the same, the pathophysiology may be different in each site⁽⁶⁾. It can be seen that with the use of antiretrovirals, the incidence and prevalence of candidiasis have been reduced considerably, since they reduce the drop in CD4 counts and can lead to very low viral loads. Despite this, there are still patients who do not receive adequate treatment and in addition to having candidiasis, they develop the persistence of disease, which indicates the need for recurrent treatments or prophylaxis with antifungals, which has, as a consequence, another important factor, i.e., resistance to antifungals.

OBJECTIVE

This study aimed to identify the prevalence of vaginal candida in a group of HIV-positive women in the city of Curitiba – Paraná, Brazil, relating it to their immune status and identifying the *Candida* sp prevalent in this population.

METHODS

This was a cross-sectional study conducted from January 2017 to January 2018, including HIV-positive women treated at the gynecology outpatient clinic of the Clinical Hospital Complex of the Federal University of Paraná (CHC-UFPR) or hospitalized at the Oswaldo Cruz Hospital in Curitiba, compared to a control group. The study was approved by the Human Research Ethics Committee (REC) of the CHC-UFPR. The inclusion criteria for the selection of the case group were non-pregnant women, HIV-positive, aged between 18 and 49 years, and who had the result of the CD4+ T cell count and quantification of viral load for a period not exceeding 3 months prior to the time of data collection. For the selection of the control group, the inclusion criteria were non-pregnant women, HIV-negative tested by Western blot (WB), aged between 18 and 49 years, and who visited for consultation at the gynecology outpatient clinic of CHC-UFPR for reasons other than vaginal discharge. All participants signed an informed consent form.

Initially, for data collection, a face-to-face interview was conducted with an approximate duration of 20 min through the use of a questionnaire formulated by the authors of the project, containing socio-demographic, epidemiological, and clinical data.

After the interview, the patients were submitted to gynecological examination to collect vaginal wall secretion through a sterile swab and subsequent seeding in Sabouraud dextrose agar medium. In addition, material was collected for the preparation of slides for microscopy and the slides were stained by Gram staining. For fungal culture, Sabouraud agar tubes were incubated at 25°C for up to 14 days. Daily macroscopic examinations of the surface of the medium were performed to observe the appearance of yeast colonies. The presence of yeast growth was confirmed by microscopic examination. The yeast colonies were inoculated on *Candida* chromogenic agar (CHROMagar *Candida*) plates for presumptive identification and verification of the purity of the isolate. The antifungigram and the identification of all isolates was performed by the VITEK® 2 automated system.

Data were evaluated quantitatively and qualitatively based on Student's t-test, non-parametric Mann-Whitney test, Fisher's test, and χ^2 test. $p < 0.05$ indicated statistical significance.

RESULTS

A total of 103 HIV-positive women were selected. One of them was excluded because of the impossibility to quantify the viral load and CD4+ T cell count. Another three women were excluded due to the impossibility of collecting vaginal secretion material as they were in their menstrual period. Two withdrew from the study, resulting in a total of 97 participants. For the control group, 112 women were included. In total, 209 patients participated in the study.

The mean age of the case group was 35.9 years and the mean age of the control group was 33.4 years ($p = 0.053$). Regarding schooling, the mean number of years of schooling was 7.7 for the case group and 11 for the control group ($p < 0.001$). More details about these data can be seen in **Table 1**.

Smoking was reported by 27.8% of HIV-positive women and only by 11.6% in the control group ($p < 0.004$). Regarding the use of illicit drugs, there was a prevalence of 33% of users in the group of women living with HIV versus 1.8% in the control group ($p < 0.001$), and

Table 1. Sociodemographic characteristics of the population studied.

Variable	Case group	Control group	p-value
Age (years)			
n	97	112	
Mean	35.9	33.4	
Median	37	34	0.053
Minimum	18	18	
Maximum	49	49	
Standard deviation	9.1	9.1	
Education (years)			
n	97	112	
Mean	7.7	11	
Median	8	11	<0.001
Minimum	0	2	
Maximum	15	20	
Standard deviation	3.5	3.7	

crack was the most prevalent drug used by 46.9% of HIV patients. Marijuana ranked second (40.6%) and cocaine ranked third (31.2%) in these patients.

The mean vaginal pH was 4.6 in the control group, while the HIV-positive group had a mean of 5.2.

Vaginal candida was present in 3.6% of the control group and 41.2% of the case group ($p < 0.001$). In women living with HIV, the most prevalent fungus was *C. albicans*, followed by *C. parapsilosis*, as shown in **Table 2**.

When women living with HIV were asked if their sexual partner was also a carrier of the virus, 82 answered the question and, among them, 32.9% accepted, 54.9% denied, and 12.2% ignored the question. Regarding the number of sexual partners, 23.7% of HIV-positive women reported having had more than 10 partners during their lives, 26.8% 5 to 10 partners, 44.3% 2 to 4, and 5.2% only one partner.

Of the 35 patients with CD4+ T-cell counts lower than 200 cells/mm³, 54.3% had candida. On the contrary, among the 62 patients with a count ≥ 200 cells/mm³, only 33.9% had the fungus ($p = 0.057$), as shown in **Table 3**.

Regarding viral load, 24.1% of the patients with undetectable quantification or less than 20 copies/mL had candida. Among those with VL between 20 and 9999 copies/mL, half were colonized, and of the patients with a viral load ≥ 10000 copies/mL, 46.9% had the fungus ($p = 0.08$), as shown in **Table 4**. When comparing the not detectable to < 20 copies/mL and the group from 20 to 9999 copies/mL alone, there was statistical significance ($p = 0.036$).

Table 2. *Candida* species found in HIV-positive patients

Species of <i>Candida</i>	n	%
<i>C. krusei</i>	5	12.5
<i>C. albicans</i>	19	47.5
<i>C. tropicalis</i>	1	2.5
<i>C. famata</i>	1	2.5
<i>C. parapsilosis</i>	9	22.5
<i>C. guilliermondii</i>	1	2.5
<i>C. glabrata</i>	4	10
Total	7	18

Table 3. Relationship between CD4+ T-cell count of women living with HIV and the prevalence of *Candida* sp.

T CD4+ (cells/mm ³)	<i>Candida</i> sp		Total
	Presence n (%)	Absence n (%)	
<200	19 (54.3)	16 (45.7)	35
≥ 200	21 (33.9)	41 (66.1)	62
p-value	0.057		

Table 4. Relationship between the quantification of the viral load of women living with HIV and the prevalence of *Candida* sp.

Viral load (copies/ml)	<i>Candida</i> sp		Total
	Presence n (%)	Absence n (%)	
ND or <20	7 (24.1)	22 (75.9)	29
20 to 9999	18 (50)	18 (50)	36
10,000 or more	15 (46.9)	17 (53.1)	32
p-value	0.08		

ND: not detectable.

DISCUSSION

Among the epidemiological data evaluated in this study, the main ones were sexual behavior and lifestyle habits. In the case of HIV-positive patients, it was possible to observe risk behaviors, such as the presence of multiple partners throughout life, which may have contributed to the acquisition of HIV. Despite Reed et al.⁽⁷⁾ stated that although sexual behaviors are associated with many genital infections, it would not be possible to clearly define the role of sexual variables as risk factors for VVC. Our data contradict this statement, as they establish a relationship between the presence of candida and risky sexual behavior. In addition, from the acquisition of HIV, the patient can develop candidiasis, not only vaginal, but also oral and esophageal, to the extent that there is compromise of the immune system.

In general, lifestyle habits are paramount in the development of pathologies. In particular, tobacco is considered the leading cause of preventable morbidity and mortality, affecting the entire organism⁽⁸⁾. Moreover, other risk behaviors in addition to those already mentioned, such as drug use, are also important factors for the acquisition of HIV⁽⁹⁾. As demonstrated in our study, the prevalence of both smoking and the use of illicit drugs was significantly higher in the group of women living with HIV, and there may be an association between smoking and development of candida in these women.

Women living with HIV, when left untreated, have vaginal candida more frequently and in a more chronic and persistent manner, in addition to responding poorly to antifungals⁽¹⁰⁾. In this study, 40 women with HIV (41.23%) had vaginal candida, a figure close to that found by Apalata et al.⁽¹¹⁾ in their sample of 97 women, of whom 52 (53.6%) had VVC. Due to the consequences of the colonization of *Candida* sp in women living with HIV, Sobel⁽¹²⁾ reports that the U.S. Centers for Disease Control issued a warning about the importance of performing serology for HIV in adult women with persistent vaginal candida, which has created a lot of criticism, since completely healthy women who are HIV-negative can also be colonized by vaginal candida, especially when exposed to risk factors such as sexual activity, pregnancy, diabetes, use of oral contraceptives, antibiotics, and a history of symptomatic vaginal candida. However, in this study, only 4 of 112 (3.57%) women in the control group had colonization by the fungus, characterizing a much lower percentage when compared to the literature, which shows the presence of *Candida* sp in about 10–20% of HIV-negative, non-pregnant women⁽¹²⁾.

Regarding the prevalence, *C. albicans* is the fungus that most causes vulvovaginitis; however, there are also other fungi, such as *C. tropicalis*, *C. glabrata*, *C. krusei*, *C. parapsilosis*, *C. kefyr*, and *C. lusitaniae*, which appear on a smaller scale, related to this pathology⁽¹³⁾. Generally, these species are only colonizers of the vaginal flora, but in some situations they can increase their virulence and cause symptoms, as in the case of women with HIV, due to the impairment of their immune status. Thus, many studies focus on the study of the prevalence of different species of candida in the vaginal environment in this group of women. According to the Baddie et al.⁽¹⁴⁾ study in Iran, the most common species that appear as colonizers in patients with HIV are *C. albicans*, *C. glabrata*, *C. dubliniensis*, *C. krusei*, *C. kefyr*, *C. parapsilosis* and *C. tropicalis*. In the present study, *C. albicans* was the most prevalent and found in 47.5% of women with HIV/AIDS, as also evidenced

in the study by Baddie et al.⁽¹⁴⁾ However, the second most prevalent fungus in this study was *C. parapsilosis*, disagreeing with the literature that shows *C. glabrata* occupying this position, being present in 22.5% of women. Seeniammal et al.⁽¹⁵⁾, who included women with and without HIV in their research, also had *C. glabrata* ranked second among the species that cause VVC. However, another study carried out in Brazil, in Salvador-Bahia, comparing HIV-positive and HIV-negative women, showed *Candida parapsilosis* as the second most prevalent species in the group without HIV, behind only *C. albicans*. In the HIV group, the prevalence of *C. parapsilosis* was the same as *C. glabrata*, ranking second⁽¹⁶⁾. Thus, there may be a relationship between the prevalence of these species and the territory studied.

In a study in Argentina, it was noticed that the HIV-1 interacts directly with candida and can potentiate its virulence, causing severe morbidity in these patients⁽¹⁷⁾. When considering the high frequency of vaginal candida among women living with HIV, immunodeficiency immediately comes to mind. In a study conducted in 1995, Shifrin et al.⁽¹⁸⁾ showed the relationship between candida infection and patients' CD4+ T-cell levels. Those with CD4+ T-cell counts of less than 200 cells/mm³ had an increase in vulvovaginal candidiasis by 8.2 times. The same conclusion was reported by Apalata et al.⁽¹¹⁾, who evaluated a significant increase in symptomatic vaginal candidiasis in HIV-positive women with CD4+ T-cells below 200 cells/mm³. Similarly, in this study, of the 35 patients with CD4 counts less than 200 cells/mm³, 54.3% had candida and 45.7% did not. Of the patients with CD4 counts greater than 200 cells/mm³, 33.9% had the fungus and 66.1% did not. Therefore, it is seen that the CD4+ T cell count appears to interfere with the pathogenesis of candida, as the comparison between the two groups revealed a p-value very close to that considered statistically relevant in the study (p=0.057). Apalata et al.⁽¹¹⁾ showed that viral load is also quite important in the prevalence of vulvovaginal candidiasis. When it shows up above 10000 copies/mL, the risk is quite high. In the present study, 50% of the women with candida had a viral load between 20 and 9999 copies/mL, and 24.1% had an undetectable viral load or less than 20 copies/mL. Most women were not included in the 10000 copies count, but it was statistically evident that the higher the viral load, the greater the chance of having VVC.

Strengths

This study provides a deeper understanding of the characteristics of the studied population related to the prevalence of *Candida* sp and reinforces the importance of controlling viral load and maintaining adequate levels of CD4+ T cells in patients with HIV in reducing morbidity and complications related to immunodeficiency.

Limitation

Other risk factors associated with *Candida* sp infection, such as comorbidities like diabetes mellitus, and the use of oral contraceptives and antibiotics, were not evaluated in this study and, although unlikely considering the statistical significance between the two groups, they may interfere with the results related to the prevalence of vulvovaginal candidiasis.

CONCLUSION

C. albicans and *C. parapsilosis* are the most prevalent species found in women with vulvovaginal candidiasis. Patients living with HIV had a significantly higher prevalence of VVC than women who were HIV-negative. Low levels of CD4+ T cells appear to be associated with a higher occurrence of candida, as well as elevated viral loads.

Approval by the Human Research Ethics Committee

The study was approved by the Human Research Ethics Committee (REC) of the CHC-UFPR.

Participation of each author

SR: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Software, Visualization, Writing. ACPGS: Data curation, Investigation, Resources, Validation. IG: Investigation, Validation, Visualization, Writing – original draft. MVFK: Investigation, Validation, Visualization, Writing – original draft. ESFC: Data curation, Investigation, Resources. MHA: Data curation, Investigation, Resources. VO: Investigation, Validation, Visualization, Writing – original draft. NSC: Conceptualization, Formal analysis, Investigation, Methodology, Project administration, Resources, Supervision.

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Conflict of interest

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Address for correspondence:**NEWTON SÉRGIO DE CARVALHO**

Rua Saldanha Marinho, 1422, apto 801

Curitiba (PR), Brazil

CEP: 80430-160

E-mail: newtonsc@gmail.com

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