

Clinicomycological study of vulvovaginal candidiasis

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Abstract

Background: Vaginal candidiasis is one of the most common vaginal infections in women of reproductive age group caused by *Candida* species of fungi, a normal inhabitant of vagina. **Aims:** The aim is to study the culture characteristics, species prevalence, and drug sensitivity of vulvovaginal candidiasis (VVC) among females with genital pruritus and discharge per vaginum who are proved to have *Candida* infection by a vaginal smear study. **Materials and Methods:** A prospective study was done on female patients aged 18 years attending the Sexually Transmitted Diseases Clinic of the Department of Dermato-Venereo-Leprology (DVL), Tirunelveli Medical College, with complaints of genital pruritus and vaginal discharge over a period of 1½ years from 2013 to 2015. After clinical examination, vaginal discharge was smeared for wet mount, potassium hydroxide mount, and Gram stain. Those with pseudohyphae were chosen for culture, speciation, and drug sensitivity. **Results:** The most common age group affected was 18–35 years. The most common predisposing factor was HIV infection-induced immunosuppression (48.7%), followed by antibiotic usage and diabetes mellitus. Clinical presentation of VVC was similar in HIV-positive and HIV-negative patients. Recurrent VVC makes up 12% of total cases with most common species being *Candida albicans* whereas *Candida glabrata* among the non-*C. albicans*. Drug sensitivity pattern of *C. albicans* showed the highest sensitivity to nystatin 85.7%, followed by triazoles (75%–85%) with ketoconazole being more resistant (40%–60%), and was similar in *C. albicans* and non-*C. albicans*. **Limitations:** Exclusion of unmarried and pregnant women and lack of follow-up were limitations in our study. **Conclusions:** According to our study, elimination of predisposing factors, speciation of fungus, and treatment based on drug sensitivity pattern will reduce the incidence of VVC.

Key words: *Candida*, fungal infection, vaginal discharge

INTRODUCTION

Vaginal Candidiasis (vaginal thrush) is the most common fungal infection of the vulva and vagina affecting nearly 75% of adult women during their lifetime.^[1] It is caused by *Candida* species and is the second most common cause of vaginitis after bacterial vaginosis. *Candida albicans* constitutes 85%–90% of cases. *Candida* is a normal inhabitant of oral cavity, gastrointestinal tract, and vagina and is a true opportunistic pathogen. The genus *Candida* is comprised of more than 200 species. The medically significant *Candida* species include *C. albicans*, *Candida glabrata*, *Candida tropicalis*, *Candida*

krusei, *Candida parapsilosis*, *Candida lusitanae*, *Candida kefyr*, *Candida stellatoidea*, *Candida guilliermondii*, and *Candida dubliniensis*.^[2] In recent years, drug-resistant non-*C. albicans* species are emerging. Over-the-counter medications favor infection by drug-resistant species. Hence, speciation and drug sensitivity of vulvovaginal candidiasis (VVC) was done, and the results were analyzed.

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Access this article online

Quick Response Code:	Website: www.ijstd.org
	DOI: 10.4103/ijstd.IJSTD_49_18

How to cite this article: Seeniammal S, Selvakumar M, Nirmaladevi P. Clinicomycological study of vulvovaginal candidiasis. Indian J Sex Transm Dis 2021;42:57-61.

Submitted: 27-Jun-2018

Revised: 11-Dec-2018

Accepted: 30-Dec-2019

Published: 31-Jul-2020

Aims and objectives

This study was done in adult females in the Department of Dermato-Venereo-Leprology (DVL) in our tertiary care hospital with the following objectives.

- To diagnose VVC among female patients with genital pruritus and discharge per vaginam by clinical and laboratory methods including wet mount, 10% potassium hydroxide (KOH) mount, and Gram staining
- To identify the culture characteristics and species prevalence of *Candida* species
- To study the drug sensitivity pattern of VVC those who were proved to have *Candida* infection by a vaginal smear study.

MATERIALS AND METHODS

This is an open prospective study which was conducted in the Department of DVL after obtaining clearance from the Ethical Committee over a period of 1½ years.

Inclusion criteria

1. All female patients aged more than 18 years with complaints of genital pruritus and discharge per vaginam attending the STD clinic
2. Those cases proved to have *Candida* infection by vaginal smear for wet mount, KOH, and Gram stain in the laboratory.

Exclusion criteria

1. Female patients aged less than 18 years
2. Patients already treated with antifungals
3. Vaginal smear negative for *Candida*
4. Pregnant women.

Oral and written consent in their local language was obtained before starting the study including consent for photographs. Clinical history regarding the duration of vaginal discharge, number of recurrences per year, extramarital contact, comorbidities such as diabetes, drug history, and contraceptive usage was taken.

A thorough genital examination regarding sites involved, nature of the discharge (colour and consistency), and surrounding skin was done. Speculum examination was also done and vaginal discharge was smeared in three separate slides. Wet mount, KOH mount, and Gram staining were done. The patients whose vaginal smear showed candidal pseudohyphae as elongated structure with cell wall constrictions in KOH mount [Figure 1] and Gram-positive rods in Gram staining were chosen for the study of culture, species identification, and drug sensitivity. The vaginal swabs were inoculated in Sabouraud dextrose agar and incubated at 37°C for

48 h. The candidal isolates were inoculated in the CHROMagar, and various species were identified based on the pigmentation [Figure 2-4]. All the isolates were subjected for antifungal susceptibility testing for fluconazole, itraconazole, voriconazole, clotrimazole, nystatin, miconazole, and ketoconazole by disc diffusion method. The basic investigations such as blood sugar, urea, creatinine, urine routine, and complete hemogram were done to look for precipitating factors. The cases were treated with antifungals according to the sensitivity and followed up after the 1st and 2nd weeks.

RESULTS

The most common age group affected was 18–35 years. The most common predisposing factor was HIV infection-induced immunosuppression (48.7%), followed by antibiotic usage and diabetes mellitus. Clinical presentation of VVC was similar in HIV-positive and HIV-negative patients.

Recurrent VVC (RVVC) makes up 12% of total cases with most common species being *C. albicans* whereas *C. glabrata* among the non-*C. albicans*. Drug sensitivity pattern of *C. albicans* showed the highest sensitivity to nystatin 85.7%, followed by triazoles (75%–85%) with ketoconazole being more resistant (40%–60%), and was similar in *C. albicans* and non-*C. albicans*.

DISCUSSION

The speciation of *Candida* and non-*Candida* organisms in our study was consistent with literature. Our study revealed the highest frequency of vaginal candidiasis in the age group of 18–35 years (55%) which was similar to literature. The least frequency of around 6.3% is observed in menopausal age group.^[3,4] Among the study group, 97% were symptomatic patients. All had itching of the vulva and vagina [Table 1]. HIV-negative and HIV-positive patients had more or less similar symptoms. However, severe forms of VVC were more common in HIV-negative patients infected with non-*C. albicans* species.

In our study, HIV infection was the major predisposing factor [Table 2]. Out of 80 cases, 39 were HIV positives. VVC was one of the most common opportunistic fungal infections in HIV-positive women. This was found to be because of depressed cell-mediated immunity, followed by diabetes mellitus, prolonged antibiotic use, IUCD, etc.^[5-7]

The genital coinfections which occurred in higher frequencies were bacterial vaginosis (6.25%), trichomonas vaginalis (5%), herpes genitalis (3.75%), and genital warts (1.25%) in decreasing order of frequency. Twenty-two women (27.5%) had no associated infections with HIV being the most common association.^[8]

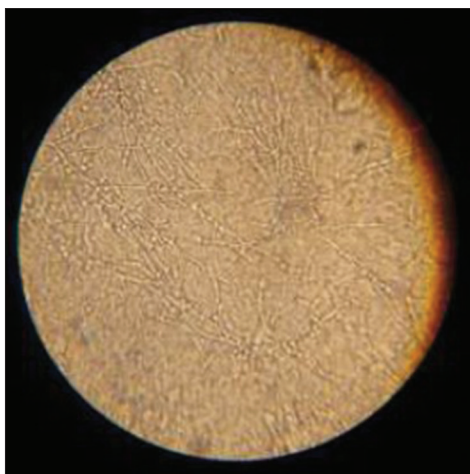


Figure 1: Potassium hydroxide mount of vaginal smear with branched hyphae and cysts



Figure 2: HiCrome agar media showing smooth green-colored wet colonies of *Candida albicans*



Figure 3: HiCrome agar media showing pink-colored wet colonies of *Candida glabrata*



Figure 4: HiCrome agar media showing metallic blue-colored colonies of *Candida tropicalis*

We observed that the most prevalent species was *C. albicans* (82%) and 18% were non-*C. albicans* [Tables 3 and 4]. We isolated nine cases of non-*Candida* species, of which four (8%) belonged to *C. glabrata*, three (6%) *C. tropicalis*, and two (4%) *C. krusei*. Reports from Mahmoudi Rad *et al.* and Fan *et al.*, have also documented similar results.^[9] In our study, *C. glabrata* was most common, followed by *C. tropicalis* and *C. krusei*. The emphasis on non-*C. albicans* was very essential because of drug resistance. Various study reports done by Study reports done by Redondo Lopez *et al.* was similar to our results.^[9,10] Hence, speciation by reliable culture methods is beneficial in the diagnostic aspect.

Over the past few years, there has been increasing incidence of primary and acquired resistance to azoles in *Candida* species. Some studies documented that the *in vitro* resistance to antifungal medication was common for non-*C. albicans*.^[11,12] The rate of fluconazole resistance varies from 5% to 25% with high resistance

in ketoconazole and itraconazole (5%–56%). Similar observations were noted in our study [Table 5] which revealed the highest susceptibility to nystatin (90%), followed by fluconazole (82%), itraconazole (80%), voriconazole (75%), and clotrimazole (75%). Ketoconazole and miconazole were less sensitive. Regarding resistance pattern, ketoconazole (61%) was more resistant, followed by miconazole (47%). We also compared the susceptibility of *C. albicans* and non-*C. albicans* drug sensitivity. For *C. albicans*, sensitivity to nystatin (87.8%) was high, followed by itraconazole (85.3%), fluconazole (82%) clotrimazole (75%), and voriconazole (75%). Ketoconazole showed high-level resistance. Regarding non-*C. albicans*, three out of four (75%) *C. glabrata* showed sensitivity to fluconazole, voriconazole, and miconazole and only 25% were resistant to fluconazole. Two cases were sensitive (50%) and two cases (50%) were resistant to Itraconazole. Drug sensitivity in *C. tropicalis* showed all the three cases (100%) to be sensitive to fluconazole, itraconazole, and nystatin, and one case was resistant to

Table 1: Analysis of clinical pattern of vulvovaginal candidiasis

Clinical features	Number of patients	HIV status		Species	
		Positive	Negative	<i>Candida albicans</i>	Non- <i>Candida albicans</i>
Asymptomatic cases	2	1	1		
Itching of vulva	78	38	40		
Scanty discharge	19	6	13		1
Profuse curdy white precipitate	51	29	25	43	8
Soddening of vulva	2	2			
Maceration of vulva, adjacent sites	8	2	6	6	2
Satellite pustules	1	1			
Erosion, inflamed fissuring	6	1	5	3	3

clotrimazole and miconazole. Two cases of *C. krusei* showed sensitivity to nystatin. Azoles (fluconazole, itraconazole, and voriconazole) were only 50% sensitive. Both the cases were resistant to miconazole. While comparing our results with other studies done on drug susceptibility pattern, all the three non-*C. albicans* species showed the highest sensitivity to nystatin. Out of nine cases of non-*C. albicans*, all were sensitive to nystatin. 66%–78% sensitivity was observed to fluconazole, voriconazole, clotrimazole, and itraconazole. Ketoconazole showed the least sensitivity (55%). Further analysis of drug sensitivity pattern in large study group is necessary. Our study showed similarity with a study done by Panchal *et al.* in Western India.^[13,14]

We compared the drug susceptibility pattern of *C. albicans* and non-*C. albicans* in this study which showed a similar pattern of sensitivity and resistance in both groups. Nystatin scored the highest sensitivity, followed by fluconazole, itraconazole, and voriconazole. Ketoconazole showed the highest resistance in both groups.

Limitations

Unmarried women were excluded in the study as speculum examination should be avoided in them. Speculum examination was avoided in pregnant women as organisms were identified in them from secretions from vulva itself. Follow-up was not analyzed for our patients as our study confined to clinicomycological study only.

CONCLUSION

VVC is common among women in the reproductive age group. Species identification by culture methods showed *C. albicans* to be the most prevalent species including the HIV-positive patients in this study. Fluconazole, itraconazole, and voriconazole were sensitive in both *C. albicans* and non-*C. albicans* infections. Nystatin was found to be a highly sensitive drug, followed by triazoles. Ketoconazole and miconazole were highly resistant to both *Candida* and non-*C. albicans* species. According to our study results, elimination of predisposing factors and ensuring good immunity with identification of causative fungal species and treatment

Table 2: Analysis of predisposing factors for vulvovaginal candidiasis

Predisposing factors	Number (%)
Antibiotic usage	8 (10)
Intrauterine devices	2 (2.5)
Immunosuppressive drugs	2 (2.5)
Diabetes mellitus	6 (7.5)
HIV	39 (48.7)

Table 3: Identifying species prevalence in the study group

Species	Number of patients (%)
<i>C. albicans</i>	41 (82)
<i>C. glabrata</i>	4 (8)
<i>C. krusei</i>	2 (4)
<i>C. tropicalis</i>	3 (6)

C. albicans: *Candida albicans*, *C. glabrata*: *Candida glabrata*, *C. krusei*: *Candida krusei*, *C. tropicalis*: *Candida tropicalis*

Table 4: Prevalence of *Candida* and non-*Candida* species in HIV-positive patients

Species	HIV positive (%)	HIV negative (%)
<i>C. albicans</i>	22 (53.6)	19 (46.4)
Non- <i>C. albicans</i>	3 (33.3)	6 (66.7)

C. albicans: *Candida albicans*

Table 5: Analysis of drug sensitivity pattern in vulvovaginal candidiasis

Drugs	Sensitive (%)	Resistant (%)
Fluconazole	41 (82)	8 (16)
Itraconazole	40 (80)	8 (16)
Nystatin	45 (90)	3 (6)
Clotrimazole	37 (74)	6 (12)
Voriconazole	28 (56)	15 (30)
Miconazole	26 (52)	13 (26)
Ketoconazole	20 (40)	23 (46)

according to the drug sensitivity pattern will reduce the incidence of VVC.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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