Original Article

A prospective observational study on species differentiation and antifungal susceptibility pattern in patients with genital candidiasis

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Abstract

Background: Candidial balanitis, balanoposthitis and vulvovaginitis can be diagnosed by direct microscopy, culture and treated with antifungals. Resistance to antifungals is emerging. Hence, we conducted a study to identify the causative species and antifungal susceptibility. **Aim:** To observe the species differentiation and antifungal susceptibility pattern in patients with genital candidiasis. **Materials and Methods:** A prospective observational study was carried out that included 54 patients of age group (18-60 years) diagnosed clinically and direct microscopically (KOH) for genital candidiasis. Culture was done using Sabouraud dextrose agar. Species identification and antifungal susceptibility were tested. Descriptive data were expressed in the form of frequency and percentage. **Results:** Out of 54 patients, 41 had culture positive candidiasis. Among the isolated species, 68.3% were *Candida albicans* (28/41) and 31.7% were non- *albicans Candida spp*. Among non-*albicans Candida* species (13/41), *Candida glabrata* (19.5%), *Candida tropicalis* (7.3%), *Candida guilliermondii* (2.4%), *Candida parapsilosis* (2.4%) were identified. Antifungal susceptibility was tested for fluconazole (FLU), clotrimazole (CLTZ), itraconazole (ITZ), ketoconazole (KTZ), voriconazole (VOR), amphotericin-B (AMPH-B). Except *C. glabrata* and *C.parapsilosis*, all other species were sensitive to all tested antifungals. All isolated species were sensitive to KTZ, VOR, AMPH-B, and CLTZ. Nearly 22% of isolates were resistant to fluconazole. **Conclusion:** *C. glabrata* causes complicated, severe recurrent vulvovaginitis which is fluconazole resistant. Drug sensitivity prior prescribing antifungal agent identifies appropriate drug, decreases patient's disease morbidity and cross resistance.

Key words: Antifungal resistance, genital candidiasis, non *albicans Candida spp*

Introduction

Genital candidiasis includes candidial balanitis, balanoposthitis and candidial vulvovaginitis. It is a superficial mycotic infection caused by yeast like fungus, Candida species. Although 200 species of Candida are known to exist, only 15 are considered to be pathogenic. *Candida albicans* is the most common causative species. Other non- *albicans Candida spp.* are also implicated in causing genital candidiasis. Unlike other fungal organisms, Candida can affect both immunocompetent and immunocompromised people. It is diagnosed based on clinical features, direct microscopy and culture. Even though the drug of choice remains fluconazole, in recent years, drug resistant Candida species have emerged. So, appropriate antifungal agent should be chosen with antifungal susceptibility testing. This study was done to observe the species differentiation and antifungal susceptibility pattern in patients with genital candidiasis.

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Materials and Methods

This study is an open label prospective observational study which was conducted in the department of dermato-venereo leprology in a tertiary care hospital between April 2021 to June 2022 after obtaining institutional human ethics committee approval.

All sexually active males and females within the age group 18-60 years diagnosed clinically and/or by direct microscopy were included in the study.

Children and adolescents of both sexes, pregnant and lactating females and patients who used a course of antifungal within a period of 1 month were excluded.

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Those patients satisfying inclusion criteria were enrolled in the study, and the specimen was collected and subjected for 10% Potassium hydroxide mount (KOH) and sent for culture and further analysis to the microbiology lab. The specimen was inoculated in Sabouraud dextrose agar at appropriate conditions (incubated at 37 and 25° C). Germ tube test and urea hydrolysis test were performed. Those specimens with candida growth were further subjected to speciation via inoculation into HI chrome and TRM (tetrazolium reduction medium) agar overnight at 37° C for 48 hours. Antifungal susceptibility testing was performed by modified Kirby-Bauer disc diffusion method as per CLSI (Clinical laboratory and standard institute) guidelines M44-A2. Control strain used for antifungal susceptibility was *Candida parapsilosis* ATCC (American type culture collection) 22019. Antifungals tested were fluconazole (FLU), itraconazole (ITZ), ketoconazole (KTZ), voriconazole (VOR), clotrimazole (CLTZ), amphotericin-B (AMPH-B) and potencies of the discs are 25 µg, 10 µg, 10 µg, 1 µg, 10 µg and 100 units, respectively. Descriptive data were analysed and presented in terms of frequency and percentage.

Results

A total of 54 patients were selected and enrolled in the study; among them, 41 had positive candidial infection by culture. Among 54 patients, 17 were immunocompromised (diabetes mellitus, long term immunosuppressant intake). Among those 17 patients, 15 patients had culture positive candidial infection. Out of the 41 patients, 11 were males and 30 were females. Among the enrolled patients, 47 had positive direct microscopy and seven (7) had negative direct microscopy (10% KOH) [Table 1]. Germ tube test was performed and was positive for all *C. albicans* isolates. Urea hydrolysis test was negative for all isolates. Among the patients with culture positive candidiasis, 28 (68.3%) patients had *Candida albicans* isolates and 13 (31.7%) patients had non *albicans Candida* isolates.

Among the non *albicans Candida spp*. isolates, species observed in the order of frequency were *Candida glabrata* (19.5%), *Candida tropicalis* (7.3%), *Candida parapsilosis* and *Candida guilliermondii* (2.4% each) [Table 2]. Out of 41 patients, 32 culture positive cases were sensitive to all tested antifungal drugs. Species that were sensitive to all antifungal drugs were *C. albicans* (28/41), *C. tropicalis* (3/41) and *C. guillermondi* (1/41). Nine culture positive cases were resistant to fluconazole (FLU) among them one patient had isolate which is resistant to both fluconazole and itraconazole (ITZ). Fluconazole resistant isolates were *Candida glabrata* (8/41) and *Candida parapsilosis* (1/41). In one patient, isolated *C. glabrata* species was resistant to both fluconazole and itraconazole. Antifungal susceptibility pattern has been summarized in Table 3.

Altogether, fluconazole sensitive species were about 78.1% and resistant species were about 21.9%. Among the isolated species, all were susceptible for KTZ, VOR, CLTZ, and AMPH-B. About 13.9% were resistant to fluconazole alone and 1.9% were resistant to both fluconazole and itraconazole.

Table 1: 10% potassium hydroxide and culture growth

10% KOH	Culture	
	Positive	Negative
Positive	41	6
Negative	0	7

KOH=Potassium hydroxide

Table 2: Frequency and percentage of individual Candida species among culture positive cases

Candida species	Frequency (%)
C. albicans	28 (68.3)
C. glabrata	8 (19.5)
C. tropicalis	3 (7.3)
C. parapsilosis	1 (2.4)
C. guilliermondii	1 (2.4)
Total	41

Discussion

Candidial infections involving external genitalia accounts for considerable morbidity in both sexes. Intense pruritus in candidial infection affects the quality of life. Lately, non *albicans Candida* have been implicated in causing complicated and recurrent candidiasis. Azole resistance is becoming common among non *albicans Candida spp*. and *C. albicans* species. This emphasizes the need for species isolation and antifungal susceptibility testing is important for treatment. KOH has 60-70% sensitivity and hence it can be used as a screening test for the diagnosis of candida infections. In our study, direct microscopy in the form of 10% KOH mount was done before culture testing. Among the 54 patients enrolled, 47 were positive for *Candida* elements in 10% KOH mount. Among 47 KOH positive patients, 41 turned out to be culture positive for candidiasis. Similar discordance in culture and KOH, was noted in a similar study and the causes attributed were presence of non viable yeasts and false positive KOH test.[1] Among the enrolled patients, eight patients had taken over the counter medications. Among them, three patients had taken oral antibiotics. Details of medication were not known for the other five patients. Six KOH positive culture negative patients were females. Four had pruritus vulva with few labial excoriations while the other two had vulval pruritus along with minimal scanty whitish discharge per vaginum. Two of those patients were immunocompromised, three were on prior antibiotic therapy while the other one did not have any comorbidity. About 20%-30% pre-menopausal women have candida colonization in their vagina. Even though, pseudo hyphae and hyphae mark invasion and infection, patients with only yeast cells in 10% KOH mount were also selected and proceeded with culture testing. This is because, unlike *C. albicans* and *C. tropicalis*, *C. glabrata* does not produce pseudo hyphae or hyphae as per Fidel *et al*.[2] So in order to minimize error of missing out on *Candida glabrata*, KOH with budding yeasts were also selected and subjected to culture testing. As with Bonifaz *et al*.,[3] Mahmoudi *et al*.,[4] and Spinillo *et al*.,[5], predominant species isolated in our study was *C. albicans* (28/41)(68.3%). Non *albicans Candida* in decreasing order isolated were *C. glabrata* (19.5%), *C. tropicalis* (7.3%), *C.parapsilosis* (2.5%) and *C. guillermondi* (2.5%). Currently, nomenclature of *C. glabrata* and *C. guilliermondii* has been changed to *Nakaseomyces glabrata* and *Meyerozyma guilliermondii*.[6]

Fan *et al*.[7] revealed 0-4.9% azole resistance among *Candida albicans* and also stated that non *albicans Candida* respond less to azole therapy. Likewise, in our study, all *C.albicans* isolated were sensitive to azoles whereas non- *albicans Candida* spp were sensitive to

Table 3: Antifungal susceptibility pattern - sensitivity and resistance assay

Antifungal susceptibility assay			
Sensitivity assay	**Resistance assay**	**Frequency (number of isolates)**	**Percentage**
Sensitive to all 6 antifungals	Not resistant to any antifungal	32	61.1
	C. albicans	28	
	C. tropicalis	3	
	C. guilliermondii	1	
Sensitive to VOR, KTZ, CLTZ, AMPH-B, ITZ	Resistant to fluconazole	8	13
	C. glabrata	7	
	C. parapsilosis	1	
Sensitive to VOR, KTZ, CLTZ, AMPH-B	Resistant to fluconazole, itraconazole	1	1.9
	C. glabrata	1	

ITZ=Itraconazole; KTZ=Ketoconazole; VOR=Voriconazole; AMPH-B=Amphotericin-B; FLU=Fluconazole; CLTZ=Clotrimazole; *C. albicans=Candida albicans*, *C. glabrata=Candida glabrata*, *C. tropicalis=Candida tropicalis*, *C. parapsilosis=Candida parapsilosis*, *C. guilliermondii=Candida guilliermondii*

some but not all azoles. Fluconazole resistance was about 21.9% total and was observed only among non- *albicans Candida* species like *C. glabrata* and *C. parapsilosis* isolates. Resistance to both fluconazole and itraconazole has also been observed by Saftar *et al*. in their study in which he stated that patients with Human Immunodeficiency Viral (HIV) infection and underlying malignancy may have higher frequencies of fluconazole and itraconazole resistant *Candida glabrata* strains that can either be associated with colonization or invasive disease.[8] In our study, one of the *Candida glabrata* isolates in a previously healthy female was resistant to both fluconazole and itraconazole, which is an uncommon finding. In a study by Song *et al*. from South Korea that two of the five *C. glabrata* isolates tested were resistant to fluconazole. But all five isolates were resistant to itraconazole.[9] In one study,[10] two *Candida glabrata* isolates were resistant to both FLU and ITZ but the isolates were susceptible to VOR similar to our study. Cell wall adhesins mediates biofilm formation in *C. albicans* and *C. glabrata* species. Ability to form biofilms are implicated for their survival mechanisms.[11] In our study, *C. parapsilosis* was also resistant to fluconazole. Persister cells in biofilms has been implicated in biofilm resistance.[12] *Candida parapsilosis* possess persister cells which might explain drug tolerance.[11,12] Recurrent candidial vulvovaginitis is more likely attributed to *Candida glabrata* species which is resistant to fluconazole. Among the drugs tested, all the Candida isolates were sensitive to KTZ, VOR, AMPH-B, and CLTZ. This is similar to study by Bloch and Smythe[13] and Greer,[14] wherein KTZ and VOR were found to be efficacious in treating candidial infection respectively. *C. glabrata* and *Candida krusei* are intrinsically resistant to fluconazole. In patients with *C. krusei* and *C. glabrata* infection, it is necessary to do a drug susceptibility assay and to treat accordingly. Cross resistance to KTZ and ITZ has been reported in literature,[15] although in our study, resistance to KTZ was not detected.

Treating the patient with a prolonged course of FLU not only increases incidence of resistance to fluconazole but also can induce cross resistance.[16] Apart from personal hygiene, Topical antifungals like CLTZ 1% or miconazole 1% twice daily for one to three weeks in mild and fluconazole 150 mg stat orally can be opted for severe cases of candidial balanoposthitis. According to CDC guidelines 2021, for uncomplicated vulvovaginal candidiasis, oral single dose fluconazole 150mg along with short course of topical antifungals is recommended. For complicated vulvovaginal candidiasis, long courses of topical azoles along with oral fluconazole, 3 doses (day 1, 4, 7) at the dosage of 100 mg or 150 mg or 200 mg and maintenance regimen with weekly fluconazole (100mg or 150mg or 200mg) for 6 months has been recommended. In case of non-albicans candidiasis, non-fluconazole azole regimen (oral or topical for a long duration of about 1-2 weeks) has been recommended. Culture and sensitivity is prudent in case if patients are symptomatic even after treatment with topical azole or oral single dose fluconazole therapy. Patients who are not responsive to empirical fluconazole therapy, those who have partial response to single dose fluconazole, and those who are immunocompromised should be promptly referred for antifungal susceptibility testing to prevent treatment resistance and to minimize the rate of recurrences. Syndromic kit for vaginitis includes fluconazole 150mg single dose along with secnidazole 2 g single dose (green colour kit). In first trimester of pregnancy, clotrimazole vaginal pessary can be advocated.

Conclusion

Genital candidiasis is a common disease among adult females and males. Even though, fluconazole remains empirical therapy for treatment of genital candidiasis, drug resistance is on the rise. Therefore, drug susceptibility testing is recommended before administering drugs in order to avoid prescribing unnecessary resistant drugs.

Limitations of the study

Small Sample Size.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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