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Original Research Article

Is there a need to review the syndromic case management of vaginal discharge due to candida in the Indian scenario?

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ABSTRACT

Background: Vulvovaginal candidiasis (VVC) affects approximately 75% of women once in lifetime. National AIDS Control Organization has recommended Kit-2/Green (tablet secnidazole 2 gm OD stat and capsule fluconazole 150 mg OD stat) for syndromic case management (SCM) of patients with vaginal discharge since 2007. Patients are frequently revisiting the STI centre with recurrent VVC. The purpose of the study was to determine the effectiveness of fluconazole and other azoles in vulvovaginitis.
Methods: Vaginal swabs from 188 patients attending regional STI centre, at Government Medical College, Nagpur between October 2020 to June 2022 were processed. A total of 128 conventionally confirmed isolates of Candida species were tested on RPMI 1640 medium for susceptibility to azoles by E test. An MIC of ≥8 µg/ml for fluconazole and ≥1 µg/ml for itraconazole, ketoconazole and voriconazole was interpreted as resistance as per CLSI M-60.
Results: Candida species isolated were *Candida albicans*, *C. glabrata*, *C. tropicalis*, *C. parapsilosis*, *C. dubliniensis* and *C. krusei*. Candida species resistant to fluconazole, itraconazole, ketoconazole and voriconazole were 22 (17.18%), 53 (41.40%), 19 (14.84%), and 3 (2.34%) respectively. *C. glabrata* was most resistant while *C. parapsilosis* was least resistant. Voriconazole was most effective.
Conclusions: Extensive use of fluconazole in syndromic case management of vaginal discharge could be the probable reason for 17.18% resistance to fluconazole. Withdrawal of fluconazole and replacement with another antifungal azole in SCM of vaginal discharge may prevent recurrent VVC and perhaps lead to emergence of fluconazole sensitive candida.

Keywords: Fluconazole, Kit-2, Resistance, RPMI 1640, Vulvovaginal candidiasis

INTRODUCTION

In early 1990s metronidazole (200 mg) and miconazole (100 mg) were recommended by National AIDS Control Organization (NACO) for symptomatic case management of vaginal discharge.[1] Subsequently in 2007, seven prepacked colour coded drug kits for sexually transmitted infections/ reproductive tract infections (STI/RTI) under National AIDS Control Programme (NACP) were initiated for syndromic management of STI/RTI. The use of Kit-2 (Green colour) in the syndromic case management (SCM) of vaginal discharge has been recommended for vaginal discharge syndrome (VDS). The kit includes secnidazole (2 gm) and fluconazole (150 mg) and is dispensed free of cost in Suraksha Clinics.[2] For almost two decades there has been no change in the contents of Kit-2 and patients with recurrent VDS has become a frequent observation in our centre.

The reasons for recurrent infection are still unclear, use of antibiotics and oral contraceptive pills, diabetes mellitus, dietary practices, specific immune defect, sexual activities, personal hygiene and socio-demographic characteristics have been implicated as possible risk factors.[3,4] Azoles are the drug of choice for treatment of VVC; yet, resistance is increasing due to continued treatment and repeated use of Kit-2 for recurrent candidiasis.[5] Fluconazole is effective in VVC patients but emerging resistance in fluconazole in *Candida* species together with less effectiveness in *C. krusei and C. glabrata* is documented.[6] Zarrinfar et al have reported resistance to fluconazole (15.7%) and itraconazole (8.3%).[7] Other investigators from China found that the resistance rates of *C. albicans* against fluconazole and itraconazole were 16.6% and 51.5% respectively.[8] Overuse of fluconazole, particularly as prophylactic agents in routine clinical practice, may be responsible for high drug resistance. Resistance to voriconazole and other azoles are scant in literature.

Hence, we made an attempt to assess the efficacy of azoles in candida isolated from cases of VVC in the region.

METHODS

Collection and processing of samples[9]

A total of 128 non-replicate isolates of candida were obtained from 188 patients with vaginal discharge attending the STI clinic in the department of obstetrics and gynecology, Government Medical College and Hospital (GMCH), Nagpur, Maharashtra from December 2020 to December 2022 were included in the study. Two high vaginal swabs were collected from each patient and processed in Regional STI Training, Research and Referral Laboratory (RSTRRL), department of microbiology, GMCH, Nagpur. One swab was used for Gram's staining and second swab was cultured on Sabouraud's Dextrose Agar (SDA) tube supplemented with 50 mg of Chloramphenicol. Candida species were identified by Gram's staining, germ tube formation, colour on Candida Differential CHROMagar (HiMEDIA Pvt Ltd., Mumbai) and morphology by Dalmau's method on corn meal agar (HiMEDIA Pvt Ltd., Mumbai).

Study design

It was an analytical cross-sectional study with groups and their comparisons.

Sample size

Samples from 188 patients (at CI 95% and absolute precision 5) were collected.

Inclusion criteria

Female patients presenting with sore, burning, itching in genital area, abnormal curd like vaginal discharge with or without pregnancy. Willingness of the patient.

Exclusion criteria

Unwilling patients. Repeat sample of same patients.

Determination of minimum inhibitory concentration by E-test:[9,10]

E-test was performed on RPMI 1640 (HiMEDIA Laboratories Pvt. Ltd, Thane, West Maharashtra, India) supplemented with morpholine propanesulfonic acid (MOPS) and 2% glucose as per the manufacturer's instructions

E Strips of fluconazole, voriconazole, ketoconazole and itraconazole were procured from The HiMEDIA Laboratories Pvt. Ltd, Thane, West Maharashtra, India.

The concentration gradient for fluconazole ranged from 256 to 0.016 µg/ml and for voriconazole, ketoconazole and itraconazole the range was 32 to 0.002 µg/ml.

MIC was determined from the inhibition ellipse that intersected the scale on the strip and interpretation were as per Clinical and Laboratory Standard Institute guidelines (CLSI M-60) (Table 1).[10]

Table 1: Interpretative criteria for antifungal susceptibility.[10]

Antifungal E strip	E strip potency (µg/ml)	MIC (µg/ml)		
		S	I	R
Fluconazole	0.016-256	≤2	4	≥8
Voriconazole	0.002-32	≤0.12	0.25-0.5	≥1
Itraconazole	0.002-32	≤0.12	0.25-0.5	≥1
Ketoconazole	0.002-32	≤0.12	0.25-0.5	≥1

Note: S: Susceptible, I: Intermediate, R: Resistant, MIC: Minimum Inhibitory Concentration

RESULTS

Out of 188 patients with vaginal discharge, candidiasis was diagnosed in 128 (68%) patients. *C. albicans* was the most predominant species in 59 (46.09%) patients followed by *C. glabrata* in 25 (19.53%), *C. tropicalis* in 20 (15.62%), *C. parapsilosis* in 12 (9.37%), *C. dubliniensis* in 6 (4.68%) as well as *C. krusei* in 6 (4.68%) patients.

Table 2: Resistance to antifungal azoles in *Candida albicans* and non-albicans candida.

Candida species	Fluconazole n (%)			Itraconazole n (%)			Ketoconazole n (%)			Voriconazole n (%)		
	S	I	R	S	I	R	S	I	R	S	I	R
***C. albicans* (n=59)**	46 (77.9)	7 (11.9)	6 (10.16)	44 (74.5)	2 (3.38)	13 (52)	49 (83)	3 (5.08)	7 (11.86)	57 (96.6)	-	2 (3.38)
***C. glabrata* (n=25)**	11 (44)	3 (12)	11 (44)	2 (8)	3 (12)	20 (80)	13 (52)	5 (20)	7 (28)	25 (100)	-	-
***C. tropicalis* (n=20)**	17 (85)	-	3 (15)	-	2 (10)	18 (90)	17 (85)	-	3 (15)	19 (95)	-	1 (5)
***C. parapsilosis* (n=12)**	11 (91.6)	1 (8.3)	-	10 (83.3)	1 (8.33)	1 (8.3)	12 (100)	-	-	12 (100)	-	-
***C. dubliniensis* (n=6)**	5 (83.3)	-	1 (16.6)	5 (83.3)	1 (16.6)	-	6 (100)	-	-	6 (100)	-	-
***C. krusei* (n=6)**	5 (83.3)	-	1 (16.6)	3 (50)	2 (33.3)	1 (16.6)	2 (33.3)	2 (33.3)	2 (33.3)	6 (100)	-	-

Note: S: Susceptible, I: Intermediate, R: Resistant

Table 3: Resistance to antifungal azoles in candida.

Antifungals (candida species=128)	Interpretation (n%)		
	S	I	R
Fluconazole	95 (74.21)	11 (8.59)	22 (17.18)
Itraconazole	64 (50)	12 (9.37)	53 (41.40)
Ketoconazole	99 (77.34)	10 (7.81)	19 (14.84)
Voriconazole	125 (97.65)	-	3 (2.34)

Note: S: Susceptible, I: Intermediate, R: Resistant

DISCUSSION

Vulvovaginal candidiasis (VVC) affects about 75% women at least once in lifetime and characterized by female patients presenting with sore, burning, itching in genital area, abnormal thick white vaginal discharge with or without pregnancy.[11] It is on record in the region (unpublished data) that patients treated syndromically with Kit-2 recommended for vaginal discharge are presenting with recurrent vaginal infection.

We found, resistance to fluconazole in *C. albicans* was 10.16%, in *C. tropicalis* was 15%, in *C. glabrata* was 44%, in *C. dubliniensis* and in *C. krusei* were 16.66% whereas no isolate of *C. parapsilosis* was found resistant to fluconazole in the present study (Table 2). Investigators from Iran have reported 6.5% isolates of *C. albicans* and 9.3% isolates of *C. glabrata* were resistant whereas no isolate of *C. parapsilosis* was resistant to fluconazole.[11] These results were in accordance with present study. However, Choudhary et al and Kumar et al have reported lower resistance to fluconazole in *C. albicans* in 1.9% and 4.7% isolates respectively.[12,13] Further they reported non-albicans candida (NAC) also showed lower resistance to fluconazole in 9.9% and 21.6% isolates respectively. Higher resistance for *C. krusei* (71.4%) was reported by Mukasa et al.[14]

We found, 22% isolates of *C. albicans* resistant to itraconazole. Amongst NAC, C. *glabrata* (80%), *C. tropicalis* (90%), *C. parapsilosis* (8.33%) and *C. krusei* (16.66%) were resistant to itraconazole. However, no isolate of *C. dubliniensis* was resistant to itraconazole (Table 2). Investigators from Uganda observed 20.6% of *C. albicans* resistant to itraconazole and 100% of *C. krusei* and *C. glabrata* were resistant to itraconazole.[14] Other investigators from Peshawar, Pakistan have reported itraconazole resistance in *C. krusei* (66.6%) and *C. glabrata* (43.7%), all in agreement with our findings.[15] Isolates of *C. dubliniensis* reported from Pakistan showed resistance in 72.7% which was in contrast with our findings. In India, investigators from western Rajasthan region have reported resistance to itraconazole in *C. albicans, C. glabrata, C. tropicalis* and *C. krusei* as 80%, 80%, 66.67% and 100% itraconazole respectively.[16] We found that resistance to itraconazole was highest among all antifungal azoles in Candida species as has been found by Kombade et al also.[16]

In the present study, all the Candida isolates exhibited low resistance towards ketoconazole. Only 11.86% isolates of *C. albicans*, 15% isolates of *C. tropicalis*, 28% isolates of *C. glabrata and* 33.33% isolates of *C. krusei* were found resistant to ketoconazole. However, no isolate of *C. parapsilosis* and *C. dubliniensis* were found resistant to ketoconazole (Table 2). Other investigators from China noted high rate of resistance to ketoconazole in *C. albicans* (27.7%) and NAC (56.2%) isolates.[17] Investigators from Tamil Nadu have reported 22% resistance to ketoconazole in *C. albicans* and 30% in *C. krusei*, whereas, all the isolates of Candida species showed 22.3% resistance to ketoconazole from Tamil Nadu.[18] The U.S. Food and Drug Administration (FDA) in its communication of July 2013 has limited the usages of ketoconazole oral tablets as they can cause severe liver injuries, adrenal gland problems and harmful drug interactions with other medications and therefore it should not be a first-line treatment for any fungal infection. However, the topical formulations of the drug have not been associated with such side effects.[19]

Among all the four antifungal azoles, we found good efficacy of voriconazole against VVC. In the present study, resistance to voriconazole in *C. albicans* was 3.38% and in *C. tropicalis* was 5% isolates. We found 100% susceptibility to voriconazole in *C. glabrata, C. parapsilosis, C. dubliniensis* and *C. krusei* isolates (Table 2). Investigators from Egypt have reported 10.5% and 20% resistance in *C. albicans* and *C. parapsilosis* respectively.[20] However, *C. glabrata, C. tropicalis* and *C. krusei* were 100% susceptible to voriconazole. Other investigators from Tamil Nadu have reported 10.6% resistance to voriconazole in *C. albicans* and 0% resistance to voriconazole in *C. parapsilosis*.[21]

In hospitals where laboratory facilities are available for speciation and antifungal susceptibility then treatment for VVC should follow the laboratory results as shown in Table 2 for our region. However, in hospitals where laboratory facilities are not available and treatment is syndromic based then treatment of patients with thick curdy white vaginal discharge should be based on the outcome as shown in Table 3 for our region.

In the present study, resistance of genus Candida was lowest for voriconazole 2.34% (Table 3). This result is in accordance to those reported by Milici et al and Matar et al.[22,23] We also found that resistance was highest for itraconazole 41.40% (Table 3). Our findings were in accordance with previous studies from Uganda[14] and Western Rajasthan.[16] We found 17.18% resistance to fluconazole and 14.84% resistance to ketoconazole in candida isolates (Table 3). Similar findings were reported in studies from India and Iran.[18,21]

Our findings have shown that there is a need to change the content of the recommended Kit-2 in SCM of patients of vaginal discharge. We found voriconazole effective. As fluconazole and voriconazole are both hepatotoxic, nephrotoxic and also have fetal toxicity, they are contraindicated during pregnancy. However, in non-pregnant women further trials in Kit-2 (green) recommended by NACO substituting fluconazole with voriconazole need to be conducted to authenticate the findings of the present study.

This study has some limitations. The study was based only on regional isolates of candida. There is a need to study fluconazole susceptibility in Candida isolates from different geographical regions of the country. Only then a change in the nationally recommended Kit-2 can be made to prevent recurrent VVC.

CONCLUSION

MIC was least to voriconazole in Candida species and was 0.003 µg/ml, as compared to fluconazole (0.125 µg/ml), itraconazole (0.094 µg/ml) and ketoconazole (0.094 µg/ml). Hence, voriconazole may be considered as an alternative drug to fluconazole (Kit-2/green recommended by NACO) for treating VVC to bring down resistance of candida species to fluconazole. Resistance to azoles in Candida species emphasizes the need for antifungal susceptibility testing in the management of VVC. Treatment given based on the susceptibility profile will help to prevent the recurrence of infection and treatment failure.

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