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ORIGINAL ARTICLE

Susceptibility Pattern of Various Azoles Against *Candida* Species Causing Vulvovaginal Candidiasis

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Abstract
Objectives Vulvovaginal candidiasis (VVC) is a common gynecological finding among the women worldwide. Candida species are often less susceptible to antifungal agents. Owing to this fact, in this study, we aimed at assessing the prevalence rate and antimicrobial susceptibility pattern of various azoles against Candida species causing VVC in symptomatic women.
Methods The prospective study included 217 female patients with symptoms of vaginal discharges. Specimens were characterized microscopically and were subjected to antimicrobial susceptibility testing against various azoles according to NCCLSM44 disk-diffusion method.
Results VVC was detected in 18.4 % of the cases. Based on age distribution, the highest rate of Candida infection was observed in the age group of 20–29 years (42.5 %). Antifungal susceptibility revealed that fluconazole was highly effective against Candida Species (97.2 %); on the contrary, the highest resistance was observed in the case of miconazole (63 %).
Conclusion In the current study, prevalence rate of VVC was found to be 18.4 %, and among the various azoles tested, fluconazole has the highest antimicrobial activity.

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Keywords Vulvovaginal candidiasis · Fluconazole · Miconazole

Introduction

Vulvovaginal candidiasis (VVC) is a common gynecological finding among women worldwide [1, 2]. It has been found that up to 75 % of the sexually active women have at least at a time experienced symptomatic VVC [3].

Symptoms generally include itching, burning, soreness, and abnormal vaginal discharge [4]. VVC is also known as Candidosis or moniliasis. It can be recurrent or relapsing [5]. When a women presents with four or more episodes per year, it is termed recurrent or relapsing VVC.

The only well-proven predisposing factors are pregnancy, diabetes mellitus [6], and the use of broad spectrum antibiotics [7], as well as oral contraceptive with high estrogen content [8]. Poorly supported risk factors include use of sponge, intrauterine devices (IUDS), douching and intercourse [7, 9], and diet with high glucose content [10]. The most *Candida* species have higher minimum inhibitory concentrations (MICs) to the various azole groups of antifungal agents, and infections they cause are often difficult to treat [11].

The present study was carried out to study the prevalence of *Candida* species in VVC and to find out the susceptibility of *Candida* species to various azoles (fluconazole, itraconazole, clotrimazole, butoconazole, and miconazole).

Materials and Methods

The present study was carried out from July 2010 to September 2010 at an Obstetrics and Gynecology OPD, GMC, Nagpur, India. A total of 217 women of different age groups were included in this study.

Two swabs were collected from each patient with the help of sterile cotton swabs. Specimen was collected from the vagina or cervix avoiding the contamination of other organism. Out of two swabs, one was subjected to direct smear examination, and the other was inoculated on Sabauraud's dextrose agar (SDA) and incubated at 37 °C aerobically. Direct smear examination was done by 10 % KOH preparation and gram staining.

The growth of *Candida* on SDA was confirmed based on colony morphology, gram staining, chlamydospore production, and other biochemical tests like sugar fermentation, sugar assimilation, and urease test were performed to identify the species of *Candida.* Germ tube test was used for confirmation of *Candida albicans*.

Antifungal susceptibility testing was performed by NCCLSM44 disk-diffusion method [12]. Inoculum was prepared by picking five colonies of approximately 1 mm in diameter from a 24-h-old culture of *Candida* species. Colonies were suspended in 5 ml of sterile saline, and its turbidity was adjusted visually with the transmittance to that produced by 0.4 McFarland standards.

Inoculations of test plates of a sterile Muller–Hinton + GMB (glucose and methylene blue) agar were performed with a sterile cotton swab dipped into the suspension. The dried surface of agar plates was inoculated by evenly streaking the swab over the entire agar surface.

Antifungal disks of fluconazole, itraconazole, clotrimazole, butoconazole, and miconazole were placed onto the surface of inoculated agar plates. Plates were inverted and incubated at 35 °C within 15 min after the antifungal disks were applied. The plates were examined after 20–24 h of incubation. The zone of inhibition was measured, and the result was recorded as susceptible, susceptible-dose dependent (S-DD) and resistant.

Results

Out of 217 specimens processed, 40 were found to be positive for *Candida* species. The infection was the commonest in the age group of 20–29 years (42.5 %) followed by 30–39 years (27.5 %), 40–49 years (25 %), and above 49 years (25 %) (Table 1).

Out of these 40 samples, 29 (72.5 %) were found to be *C. albicans*, and the remaining 11 (27.5 %) were non-albicans, *Candida.*

Table 1 Agewise distribution of vaginal candidiasis

Age (years)	No. of +ve samples ($n = 40$)	Percentage
<20	1	2.5
20–29	17	42.5
30–39	11	27.5
40–49	10	25
>49	1	2.5

Table 2 Percentage sensitivity of *Candida* species to various azoles

S. no.	Antifungal agent	Percentage sensitivity
1	Fluconazole	97.2
2	Clotrimazole	80
3	Itraconazole	57
4	Butoconazole	40
5	Miconazole	37

Of the *Candida* isolates tested, 97.2 % were sensitive to fluconazole followed by clotrimazole (80 %), itraconazole (57 %), butoconazole (40 %), and finally miconazole (37 %) (Table 2).

Discussion

The VVC is the second most common cause of vaginitis after anaerobic bacterial vaginosis. The VVCs reported by different researchers show the incidence rate as ranging from 9.5 to 30.43 %. The overall prevalence of VVC was found to be 18.4 % which is similar to the findings of other studies from India [13, 14] and elsewhere [15, 16].

The age range with the highest infection rate with vaginal candidiasis was 20–29 years (42.5 %) which is comparable with the study of Goh and Thrimoorthy in Singapore (55.5 %) [17].

Candida albicans remain the common isolate in the study. Incidence of *C. albicans* varies from 43.1 to 87.5 %. In the present study, the incidence was 72.5 % which is quite comparable with the studies of Jindall and Aggrawal, Amritsar (74.4 %) and Kikani (66.1 %). The incidence of non-albicans *Candida* is also very similar to the finding of Jindall and Aggrawal.

It is observed that *C. albicans* accounts for 70–90 % of VVC cases, with a recent emergence of non-albicans species (Paulitsch and Weger et al. 2006, Austria) [8]. *Candida glabrata* is the primary non-albicans species emerging in VVC, accounting up to 40 % of infection in immune-competent women.

Antifungal susceptibility testing revealed that only 2.8 % isolates of *C. albicans* were resistant to fluconazole. Similar susceptibility pattern was reported by Richter et al. [18]. A study in Brazil also reported no fluconazole resistance among 50 *C. albicans* vaginal isolates. Maximum resistance was observed against miconazole (63 %) followed by butoconazole (60 %).

In conclusion, our study provides information on antifungal susceptibility of vaginal yeast isolates in Nagpur, India. Since the majority of *C. albicans* isolates were susceptible to fluconazole, its use may be continued for empirical therapy of candidal vulvovaginitis. The use of alternative agents (like clotrimazole, boric acid, flucytosine, etc.) [19], may be considered when treating vulvovaginitis caused by non-albicans species. As only a limited number of *Candida* isolates could be tested in this study, further studies need to be performed involving a larger number of isolates to confirm the findings.

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Conflict of interest None.

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