

Phenotypic Characterization and Antifungal Susceptibility Pattern to Fluconazole in *Candida* species Isolated from Vulvovaginal Candidiasis in a Tertiary Care Hospital

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ABSTRACT

Background: Vaginal candidiasis is a common gynecological finding among women worldwide. This study was carried out to determine the prevalence of vulvovaginal candidiasis (VVC) along with speciation of *Candida* with special reference to its antifungal susceptibility pattern to fluconazole and also to evaluate the risk factors responsible for VVC in patients attending our tertiary care hospital in Puducherry, India.

Materials and Methods: This study was carried out in the tertiary care hospital in Puducherry during the period of August 2010 to September 2012. The study group consisted of 180 women between the age group of 15 to 56 years with the complaints of excessive vaginal discharge, pruritis and pain. Materials used for this study consisted of high vaginal swabs from patients with relevant history, attending Obstetrics & Gynecology department. High vaginal swabs were subjected to direct 10% KOH wet mount microscopy, Gram stain, culture onto Sabouraud's dextrose

agar (SDA) & 5% sheep blood agar and susceptibility testing to fluconazole was performed using E-test.

Results: *Candida* was isolated in 40 (22.2 %) women & these consisted of *C. albicans* 26 (65%), *C. glabrata* 9 (22.5%), *C. tropicalis* 3 (7.5%) & *C. parapsilosis* 2 (5%). Susceptibility test carried out on the 40 isolates revealed that 35 (87.5%) *Candida* isolates were sensitive to fluconazole, 3 (7.5%) were moderately sensitive and 2 (2.5%) were resistant. Thirty one percent patients had itching as the presenting complaints followed by vaginal discharge (29.4%).

Conclusion: The high frequency with which *C. albicans* was recovered in our study and its susceptibility to fluconazole supports the continued use of azole agents for empirical therapy of uncomplicated candidal vulvovaginitis in the community.

Keywords: Candidiasis, Vulvovaginitis, *Candida*, Vulvovaginal candidiasis

INTRODUCTION

Vaginal candidiasis is a common gynecological finding among women worldwide [1]. It has been found that up to 75% of the sexually active women have symptomatic vaginal candidiasis at least once [2]. *Candida* spp., mostly *C. albicans*, can be isolated in the vaginal tracts of 20 to 30% of healthy asymptomatic non-pregnant women at any single point of time and in up to 70% if followed longitudinally over a 1-year period. If the balance between colonization and the host is temporarily disturbed, *Candida* can cause disease such as VVC, which is associated with clinical signs of inflammation.

The commonest organism implicated is *Candida albicans* [3], and the predisposing factors include: hormonal fluctuations in pregnancy, luteal phase of menstrual cycle, use of oral contraceptives, and hormone replacement therapy among others [4]. VVC show acute or chronic courses and different disease patterns which can strongly affect the quality of life of the women who are concerned. Hence an understanding of the impact and spread of *Candida* infections among women in Puducherry will be of immense help in the management of gynecological problems in the symptomatic cases. Therefore a study was done in Aarupadai Veedu Medical College & Hospital to determine the various species of *Candida* causing vulvovaginal candidiasis, their resistance profiles & their predisposing factors for establishing empiric treatment protocols.

AIMS AND OBJECTIVES

To find out the prevalence of vulvovaginal candidiasis, along with speciation of *Candida*, with special reference to its antifungal susceptibility pattern to fluconazole and evaluation of the risk factors responsible for VVC in patients attending our tertiary care hospital in Puducherry, India.

MATERIALS AND METHODS

This study was carried out in the Department of Microbiology in collaboration with Dept of Obstetrics & Gynecology (OG), Aarupadai Veedu Medical College and Hospital, Puducherry, India during the period of August 2010 to September 2012. The study was started after getting the ethical clearance from the scientific research committee of the institution. An informed written consent was obtained from all the subjects. The study group consisted of 180 women between the age group of 15 to 56 years with the complaints of pruritis, pain and vaginal discharge. One hundred & eighty women with no history of vaginal discharge were included as controls. Materials used for this study consisted of high vaginal swabs from patients, with relevant obstetric and gynecological history, attending OG OPD. A questionnaire was completed with information covering complaints, nature of vaginal discharge, personal history, marital history, predisposing factors, per vaginal examination and collection of high vaginal swabs. Data collected

included name, age, complaints like vaginal discharge, pain and itching, duration of illness whether acute or chronic, any past history of presenting episode, any systemic diseases such as diabetes/hypertension/tuberculosis. Two sterile, cotton tipped swabs were used to collect specimens from lateral wall of vagina of each woman. One of the two swabs was used to determine the presence of yeast by direct wet-mount microscopy using a drop of 10 per cent potassium hydroxide solution. The other swab was used for culture onto Sabouraud's dextrose agar (Hi-Media, Mumbai, India) supplemented with 0.06 mg/ml gentamicin, with and without cycloheximide (0.5%) & 5% sheep blood agar incubated at 37°C. Species identification was done as per standard procedures [5]. Susceptibility testing to fluconazole was performed using E-test [6]. HiComb MIC test (A-strip- 256-2mcg & B-strip- 2.048-0.016 mcg). Species identification of yeast isolates were done by standard procedures including morphology, germ tube test, cornmeal agar test (Hi-Media, Mumbai, India), HiChrome agar (Hi-Media, Mumbai, India) and assimilation of various sugars.

RESULTS

Fifty six (31%) patients were having itching as the presenting complaints, 53 (29.4%) presented with vaginal discharge as the initial complaint, 28 (15.6%) patients presented with pain as the initial complaint, 24 patients (13.3%) presented itching, pain & discharge as complaints. The commonest organism isolated was bacterial in origin which constitutes 71 patients (39.4%). *Candida* species were isolated from 40 patients (22.2%). The other organisms isolated were *Trichomonas vaginalis* and Gram negative bacilli.

Pregnancy was the commonest risk factor for VVC 22(55%) followed by broad spectrum antibiotic usage 8 (20%). Other risk factors were Diabetes mellitus, usage of oral contraceptive pills and tuberculosis [Table/Fig-1]. Out of 40 isolates, 27 (67.5%) were from pregnant women. Thirty five (87.5%) *Candida* isolates were sensitive to fluconazole, 3 (7.5%) were moderately sensitive and 2 (2.5%) were resistant. The results were analyzed statistically using Chi-square method at appropriate places.

S.No	Risk Factors	Number (%)
1	Pregnancy	22(55)
2	Antibiotic usage	08(20)
3	Diabetes mellitus	06(15)
4	Oral contraceptive pills	03(7.5)
5	Tuberculosis	01(2.5)
Total		40

[Table/Fig-1]: Risk Factors for VVC

Age	Control group (%)	Patient group (%)
15-25 Yrs	36(20)	40(22.2)
26-35 Yrs	90(50)	82(45.5)
36-45 Yrs	41(23)	38(21.2)
46-55Yrs	12(6.5)	14(7.8)
>56 Yrs	01(0.5)	6(3.3)
Total	180	180

[Table/Fig-2]: Categorization of control & patient groups

S. No	Species	Number (%)
1.	<i>C. albicans</i>	26(65)
2.	<i>C. glabrata</i>	09(22.5)
3.	<i>C. tropicalis</i>	03(7.5)
4.	<i>C. parapsilosis</i>	02(5)
Total		40

[Table/Fig-3]: Distribution of *Candida* species isolated from vaginal discharge in SDA

DISCUSSION

In given study 93% normal healthy females taken as controls were between 15-45 years of age. Only 7% was in the age group of 46- 55 years [Table/Fig-2]. Various studies have reported the prevalence of asymptomatic vaginal colonization of *Candida* species as 5% to 30% [7,8]. The vaginal carriage rate of *Candida* species in asymptomatic controls in our study was 8%, which is similar to the study done by de Oliveira JM et al., [9] who reported the asymptomatic prevalence of VVC as 10%. Vaginal colonization is more frequent in diabetic women [10]. Numerous studies worldwide have shown that *C.albicans* can convert from being a commensal into a disease-causing pathogen, in response to a change in the host environment causing infections in the oral, gastrointestinal and genital tracts. Moreover as a commensal, *C. albicans* asymptotically colonizes epithelial surfaces presumably in the form of blastoconidia. In the present study Gram staining of high vaginal swab in these normal healthy controls revealed *Candida* only as a blastoconidia not as elongated hyphae or pseudohyphae. In the present study 88.9% of the women were between the age group of 15-45 years. Studies done by various authors say the incidence of reproductive tract infections in women is highest in the age group of 15-45 years and followed by a decline [11]. The reason for the high incidence in this age group includes low levels of protective cervical antibodies, increased sexual activity, and new influence of reproductive hormones that may lead to increased susceptibility to reproductive tract infections [11]. Our result is in consistent with other studies [11]. Postmenopausal women appear to be more resistant to *Candida* colonization, although the incidence of VVC rises among women using hormone replacement therapy, the prevalence of asymptomatic *Candida* declines with increasing age. In our study only in 6 symptomatic postmenopausal women *Candida* species was isolated. This reduction in prevalence of *Candida* in postmenopausal women may be due to decreased levels of reproductive hormones. High levels of reproductive hormones are generally thought to provide a better source for growth of *Candida* by inducing higher glycogen contents in the vaginal epithelial cells and also some studies say that estrogens have a direct effect on the growth of *Candida* and its adherence to the vaginal epithelium [12].

In our study, 31% patients were having itching as the presenting complaints, 29.4% presented with vaginal discharge as the initial complaint, 15.6% patients presented with only pain as the initial complaint, 13.3% presented itching, pain & discharge as complaint. Other complaints reported are dysuria, redness, dyspareunia, vaginal and vulvar erythema. Our study is in consistent with other authors [13,14].

In the present study, 74.5% of the women belonged to low socioeconomic status. Despite therapeutic advances, vulvovaginal candidiasis remains a common problem worldwide, affecting all strata of society. In our study women of lower socioeconomic strata, poor genital hygiene & illiteracy showed significantly higher incidence of VVC. The use of synthetic clothes could be contributing to VVC by increasing perineal moisture. Our study is in consistent with work done by Jindal et al.,[15].

In the present study behavioral and host-related risk factors associated with VVC and recurrent episodes were assessed. Statistically highly significant difference in incidence of VVC was observed between pregnant and non pregnant women. In the present study 55% of the females who presented with the complaints were pregnant. This may be probably due to high level of reproductive hormones during pregnancy which provides an excellent carbon source for growth of *Candida* [12] and also an increased susceptibility to infection by species of *Candida*, resulting in both a higher prevalence of vaginal colonization and a higher rate of symptomatic vaginitis. Our findings were comparable with other studies [15,16].

Significant influence was observed during use of broad spectrum antibiotics by increasing the incidence of VVC in the present study. *Candidal* vulvovaginitis is a common occurrence after systemic use of broad spectrum antibiotics [13,14]. Antibiotic agents increase vaginal yeast colonization and are thought to act by eliminating lactobacilli, thereby facilitating *Candida* to grow, adhere and germinate. The concept of interaction between lactobacilli and *Candida* includes competition for nutrients and steric interference of adherence to vaginal epithelial cells [13,14].

In our study diabetes was the third most common risk factor found in females with VVC. Many investigators have suggested that vulvovaginal candidiasis (VVC) occur more frequently in diabetics. Further, they also suggest that chronic recurring VVC may be a marker of diabetes. Several studies report increased rates of incidence of symptomatic infection are seen in diabetic women, but results are inconsistent. Potential risk factors for VVC include type of diabetes, severity, and degree of glucose control [17].

High incidence of VVC is also observed in patients on oral contraceptives, and is similar to the findings of other investigators [13,14].

In the present study, culture for *Candida* species was positive in 40 (22.2%) of 180 women attending our Obstetrics & Gynaecology OPD. This gave a prevalence of 22.2%. This study is in agreement with the work conducted by Bauters et al., [7] who isolated *candida* species in 20.1%. Mohanty et al., [14] reported 18.5% prevalence of vulvovaginal candidiasis in a community setting. In the present study the prevalence was found to be higher in the age group of 15-45 years. Out of 40 *candida* species isolated, *C.albicans* accounted for 65%, followed by *C.glabrata* in 22.5%, then *C. tropicalis* in 7.5%, *C. parapsilosis* in 5% [Table/Fig-3]. Studies conducted in various countries revealed *C. albicans* to be the most common species in women with VVC (76 to 89%), followed by *C. glabrata* (7 to 16%) [18-20]. The percentage of non *C. albicans* species associated with VVC in these countries ranged from 11% to 24%[18-20]. Some studies have reported an increasing trend in the occurrence of non-*C. albicans* species over time [20,21]. In the present study *C.glabrata* (22.5%) was the most common non albicans species isolated which is consistent with a study done by Ahmad et al.,[13] in Aligarh, India.

C.albicans isolated in the present study was lesser than the study done by other authors [22,23]. Vaginal culture is also essential for identification of various *candida* species. In the present study, although *C. albicans* (65%) predominated, nonalbicans species were found to be present in 35% of infections. Recently several authors have also reported an increase in the incidence of VVC caused by nonalbicans species of *candida* [22]. The highest proportion of nonalbicans *candida* reported is that of *C. glabrata*, which is similar to the finding of our study [21-23]. These nonalbican yeasts are relatively nonpathogenic but ultimately get selected and start appearing more frequently because of the widespread abuse of over the counter antifungals, use of single dose oral and topical azole regimens, and long term maintenance regimens of oral azoles. Therefore vaginal culture is valuable not only for identifying the species of vaginal *candida* but also for monitoring the changing trends in the microbiology of vulvovaginal candidiasis which is essential for the complete and prolonged treatment [21,22].

The antifungal susceptibility patterns to fluconazole for the 40 *candida* isolates were performed by E-test method as per standard protocols. Out of 40 isolates, 87.5% were sensitive to fluconazole, 7.5% were moderately sensitive & only 5% were resistant. Out of the 3 *candida* isolates that were moderately sensitive, 2 were *C.glabrata* & one was *C.tropicalis*. Most non-albicans *Candida* species have higher azole MICs and infections

they cause are often difficult to treat [24,25]. With multiple antifungals and varying susceptibility patterns of *Candida*, it has now become necessary to perform antifungal susceptibility testing and make reports available to the clinician for effective therapeutic outcome. Fluconazole resistance in vaginal *C. albicans* isolates is an uncommon occurrence. In our study, none of the *C.albicans* isolates were fluconazole resistant.

CONCLUSION

VVC caused by *C.albicans* is prevalent in our region. The high frequency with which *C. albicans* was recovered in our study and its susceptibility to fluconazole supports the continued use of azole agents for empirical therapy of uncomplicated *candidal* vulvovaginitis in the community. More prospective studies are needed to determine the optimal therapy for *candidal* vulvovaginitis caused by non-albicans species.

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