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.

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

Keywords: Candidiasis, Vulvovaginitis, Candida, Vulvovaginal candidiasis
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 were Trichomonas vaginalis and Gram negative bacilli.

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 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].

### Table/Fig-1: Risk Factors for VVC

<table>
<thead>
<tr>
<th>S.No</th>
<th>Risk Factors</th>
<th>Number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Pregnancy</td>
<td>22(55)</td>
</tr>
<tr>
<td>2</td>
<td>Antibiotic usage</td>
<td>08(20)</td>
</tr>
<tr>
<td>3</td>
<td>Diabetes mellitus</td>
<td>06(15)</td>
</tr>
<tr>
<td>4</td>
<td>Oral contraceptive pills</td>
<td>03(7.5)</td>
</tr>
<tr>
<td>5</td>
<td>Tuberculosis</td>
<td>01(2.5)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>40</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Age</th>
<th>Control group (%)</th>
<th>Patient group (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15-25 Yrs</td>
<td>36(20)</td>
<td>40(22.2)</td>
</tr>
<tr>
<td>26-35 Yrs</td>
<td>90(50)</td>
<td>82(45.5)</td>
</tr>
<tr>
<td>36-45 Yrs</td>
<td>41(23)</td>
<td>38(21.2)</td>
</tr>
<tr>
<td>46-65Yrs</td>
<td>12(6.5)</td>
<td>14(7.8)</td>
</tr>
<tr>
<td>&gt;66 Yrs</td>
<td>01(0.5)</td>
<td>06(3.3)</td>
</tr>
<tr>
<td>Total</td>
<td>180</td>
<td>180</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>S. No</th>
<th>Species</th>
<th>Number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>C. albicans</td>
<td>26(65)</td>
</tr>
<tr>
<td>2</td>
<td>C. glabrata</td>
<td>09(22.5)</td>
</tr>
<tr>
<td>3</td>
<td>C. tropicalis</td>
<td>03(7.5)</td>
</tr>
<tr>
<td>4</td>
<td>C. parapsilosis</td>
<td>02(5)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>40</td>
</tr>
</tbody>
</table>
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 stearic 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 nonalbacens 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 ‘broad spectrum antibiotics’ 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 Candida 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.

REFERENCES

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