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RESEARCH ARTICLE

Isolation and identification of Candida Species in Patients with Vulvovaginal Candidiasis

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Abstract

Abnormal vaginal discharge is one of the frequent complaints of women of reproductive age group. This study was carried out to determine the prevalence of vulvovaginal candidiasis(VVC) among the patients attending the tertiary care hospital with complaints of suggestive of vaginitis. This study was done in a tertiary care hospital, Chennai for a period of 1 year from January 2017 to December 2017. The study included 160 women of the age group 15 - 65 years with complaints suggestive of vaginitis. High vaginal swabs were taken and subjected to direct microscopy, cultured onto Sabouraud Dextrose Agar (SDA) and Hichrome Candida differential agar. Candida species were determined by standard microbiological methods and the results were confirmed by automated VITEK2 compact. Candida species were isolated from 56 patients which included *C.albicans* (25), *C.tropicalis* (20), *C.glabrata* (6), *C.parapsilosis*(4), *C.krusei*(1). Our study shows higher prevalence of non albicans Candida causing VVC. Hence, we recommend that the investigations up to species identification of Candida may be routinely followed in the microbiology laboratories.

Keywords: Candida species, vulvovaginal candidiasis, risk factors.

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INTRODUCTION

Fungal infections have become an alarming problem over the last ten years mainly because of global increase in the number of immunocompromised patients, who are highly susceptible to opportunistic infections, including mycoses[1]. According to a recent study, the incidence of Candidaemia is 6.9 per 1,000 ICU patients of which 7.5% received antifungal therapy[2]. Among the women, vaginal candidiasis has become a common finding worldwide and up to 75% of them have symptomatic vaginal candidiasis at least once[3]. Vulvovaginal candidiasis (VVC) is defined as signs and symptoms of inflammation of the vulva and vagina in the presence of *Candida* spp. and in the absence of other infectious etiology[4]. The clinical features of VVC include pruritus, hyperemia, vaginal discomfort and leucorrhea, burning, soreness, dyspareunia and vaginal or vulvar erythema, which may cause a problem in marital and sexual relations[5].

Candida albicans appears to be the cause for 80 to 92 percent of vulvovaginal candidiasis[6]. There is an increase in frequency of other candida species nowadays, specifically of *C. glabrata,* may be because of increased use of over-the counter drugs, long-term use of azoles, and the use of short courses of antifungal drugs[7]. The prevalence of candida in India is estimated to be 30%[8]. There are various predisposing factors of VVC, few of which are, hormonal fluctuations in pregnancy, luteal phase of menstrual cycle, use of oral contraceptives, and hormone replacement therapy[9].

Candida albicans is considered as an important fungal pathogen among humans because of its varying virulence factors that leads to candidiasis like phenotypic switching, phospholipase, proteinase and hemolytic activity. 85-90% of all cases of vulvovaginal candidiasis shows *C.albicans* followed by C.*glabrata* (5-10%), C. *tropicalis* (3-5%) and other species[10]. Though VVC is one of the most common fungal disease worldwide, the information about the distribution and etiology of it is scarcely known to us, because microbiology tests to identify the species of Candida and their antifungal susceptibility testing are not routinely performed in most of the laboratories[11]. Hence, this study is undertaken to determine the species specific prevalence and the risk factors associated with occurrence of VVC.

MATERIALS AND METHODS

This study was conducted in a tertiary care hospital in Chennai over a period of 1 year. The present study enrolled 160 patients attending the Obstetrics and Gynaecology Out patients department and Inpatients admitted in the wards with symptoms suggestive of vaginitis. The study has been approved by institutional ethical committee and Informed consent was obtained from all the subjects.

Sterile vaginal swabs were used for collection of vaginal samples from the patients. Two high vaginal swab samples were collected aseptically from the posterior vaginal fornix using speculum and posterior vaginal wall retractor. The swabs were transferred to the microbiology laboratory and processed immediately.

One of the swabs was used for Gram stain and direct wet mount microscopy using 10% Potassium hydroxide solution to determine the presence of yeast cells in the sample. The second swab was streaked on Sabouraud Dextrose agar (SDA) (HiMedia, India) plates containing chloramphenicol and incubated at 37°C for 24-48 hours. The colonies from SDA plate were subjected to Gram stain to confirm the growth of candida and were further subtyped by streaking on Hichrome Candida differential agar (HiMedia, India) and incubated at 37°C for 24-48 hours. The pigmented colonies were further examined for assimilation of various sugars and candida species were identified using standard microbiological methods. The colonies from the SDA plate were again processed in automated VITEK2 compact (bioMérieux) and the results were documented.

The colonies from SDA plate were also subjected to germ tube test to check for the production of germ tubes. Germ tube test is done by mixing 3-4 colonies in 0.5 ml human serum and incubated at 37°C for 2-4 hours and examined under microscope for the formation of germ tube. Colonies suggestive of *C.albicans* were confirmed by this germ tube test.

RESULTS

A total of 160 women with complaints suggestive of vulvovaginal infections were enrolled in the present study. Their age ranged from 15 – 65 years (table -1). In our study 51 women presented with complaints of white discharge (32%), 45 women (28%) had complaints of itching and 18 women (11%) had pain.

Table 1. Agewise distribution of the study subjects

Age in years	No. of patients (n=160)	Percentage (%)
<20	2	1.2
20-40	124	77.5
40-60	31	19.4
>60	3	1.9

Table 2. Risk factors associated with the study subjects :

Risk factor of VVC	No. of patients
Pregnancy	20
Antibiotic usage	4
Oral contraceptive pills usage	4
Diabetes	6
Tuberculosis	1

Out of the 160 high vaginal swab samples, Candida were isolated from 56 samples. The isolated candida was processed for species identification. The Various candida species isolated in the current study were shown in the table.

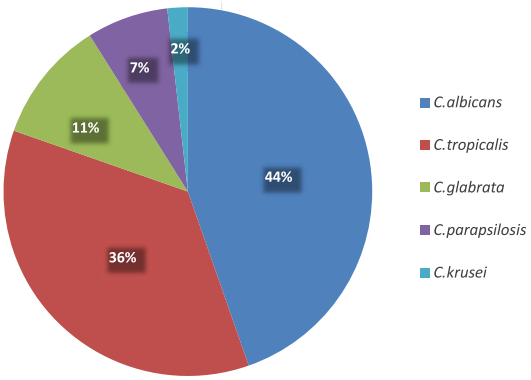

Fig. 1. Percentage wise distribution of various Candida species causing vulvovaginal candidiasis

Table 3. Species distribution of candida

Candida species	No. of isolates
C. albicans	25
C. tropicalis	20
C. glabrata	6
C. parapsilosis	4
C. krusei	1

DISCUSSION

Vaginitis is a universal problem affecting millions of women globally. Vulvovaginal candidiasis (VVC) is defined as signs and symptoms of inflammation of the vulva and vagina in the presence of *Candida* spp. and in the absence of other infectious etiology[12].

It is an established fact that *C.albicans* can convert into a disease causing pathogen from a commensal, when there is a change in the host environment[13]. The increasing antifungal resistance leading to treatment failures and added mortality warrant the need for identification of species in candida. The incidence is more common in women with diabetes where high blood sugars favour growth of candida. Other common associations for the predisposition of candida include pregnancy, antibiotic use and rarely infections like Tuberculosis.

In our study of 160 women, 56 high vaginal swabs (35%) were culture positive and grew candida species. This data is similar to reports by Kumari *et al* (30.6%), but lower than reports from Namrata Kalia *et al* (47%) and ranks second as the cause for vulvo vaginal infections[14].

Among the 56 women with positive cultures, 20 were pregnant (35%), 6 women were diabetic (11%), 4 had a history of antibiotic use (7%), and 4 were taking Oral contraceptive pills (OCP) (7%). In our study, there was significant association of positive cultures with pregnancy which could be attributed to high levels of reproductive hormones inducing higher glycogen content in vaginal epithelial cells favouring growth of candida. Some studies say that estrogens have a direct effect on the growth of Candida and its adherence to the vaginal epithelium which explain the increased incidence in women using OCPs [15,16].

The other major risk factor is diabetes where the likely reasons could be due to

uncontrolled blood sugars and also the use of unsuitable antifungal agents[17]. The use of antibiotics acts as a short term risk factor for the symptomatic vulvovaginal candidiasis due to the loss of lactobacilli and other normal flora in the vaginal surface[18].

Most patients who seek medical attention have complaints of white discharge, itching and pain. In our study 51 women had complaints of white discharge (32%) which was the major complaint. It was followed by complaints of itching which was seen in 45 women (28%) and complaints of pain by 18 women (11%). The clinical presentation was slightly different from reports by Latha ragunathan *et al*[19], where itching (31%) was the major complaint followed by white discharge (29.4%) and pain (15.6%).

In our study identification of candida species was done by conventional methods and confirmed by Vitek 2 compact. There were various candida species detected in our study with highest being *C. albicans* (44%), and non albicans (56%). Our study showed results similar to study by Kumari *et al*[12] and studies from other parts of the world where there was a higher prevalence of non albicans species[20,21,22]. This shows the increasing trend of non albicans species, which could be due to environmental variation. The increasing frequency warrants the need to identify the species and test the anti-fungal susceptibility to commonly used antifungal drugs.

The prevalence of non albicans species in our study were as follows *C.tropicalis* – 20 (36%), *C.glabrata* – 6 (11%), *C.parapsilosis* – 4 (7%), *C.krusei* – 1 (2%). Among the NAC (Non albicans Candida) *C.tropicalis* was the predominant species followed by *C.glabrata* which was similar to studies by Jayalakshmi *et al*[23] and Sundar Khadka *et al*[24], but there was a slight difference in the study by Faraji *et al*[17] where candida was isolated from diabetic women only. The predominant Non albicans species was *C.glabrata* followed by *C.tropicalis* in the study by Sundar Kadka *et al*[24]. The other species *C.parapsilosis*, *C.krusei* have been reported less frequently in patients with vulvovaginitis similar to our study[23].

Thus, this study shows a changing trend in the causative agents of VVC. Increasing emergence and spread of various non albicans candida is a major concern in the management of VVC.

CONCLUSION

Candida albicans was found to be the predominant species isolated in the current study followed by *C.tropicalis*. Yet, there is higher prevalence of non albicans candida species in the study. Hence, screening of all women with vulvovaginal infections for different species of candida would be helpful in providing better care. Thus, complete identification of causative agent of Vulvovaginal candidiasis upto species level in all the microbiology laboratories is highly recommended in order to study the emergence and spread of non albicans candida in the community. More intensive studies are needed to determine the optimal treatment of antifungal drugs for VVC caused by non albicans species.

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