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Study of vulvovaginal candidiasis in symptomatic women of reproductive age group attending tertiary care institute, Haryana province

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ABSTRACT

Background: Abnormal vaginal discharge results from a variety of infectious and non-infectious causes. Vulvovaginal candidiasis (VVC) is caused by the overgrowth of *Candida* species in the vagina and is characterized by itching, erythema and curd like vaginal discharge. Vulvovaginal candidiasis is most often caused by *Candida albicans*, however, other species of *Candida* such as *Candida glabrata, Candida parapsilosis*, and *Candida tropicalis* are emerging. **Aims and Objectives:** The current study was designed to study microbiological diagnosis and anti-fungal susceptibility testing of vulvovaginal candidiasis in symptomatic women of reproductive age group attending our tertiary care institute. **Materials and Methods:** The present study was conducted in female patients in the reproductive age group (15 to 50 years), with the complaint of excessive vaginal discharge. Vaginal swabs collected from patients with abnormal vaginal discharge were processed for direct examination of the *Candida* species. Results of the culture and antimicrobial sensitivity testing were documented. Collected data was entered in Microsoft excel sheet and analysed. Statistical analysis was done using descriptive statistics. **Results:** During study period 300 patients were included for this study after satisfying inclusion and exclusion criteria. All patients underwent microbiological study of vaginal discharge sample to diagnose vulvovaginal candidiasis. Total 300 samples were tested, out of which 18.33% (55 samples) were tested positive for candidiasis. Positive samples were studied further. In present study 26-30 years age group (25%) was most common age group followed by 31-35 years (24%). Vaginal/vulvar itch (85%), vaginal malodour (78%) and vaginal discharge (71%) were most common symptoms noted in present study. Previous history of similar complaints in past (38%), history of recurrent vulvovaginal candidiasis (16%) and antibiotic use (15%) were most common predisposing factors for vaginal candidiasis in present study. Out of 55 isolates, *Candida albicans* (65%) was most common followed by *Candida tropicalis* (24%) and *Candida glabrata* (11%). Antifungal susceptibility was best for amphotericin B followed by clotrimazole, miconazole and least for fluconazole. **Conclusion:** Vaginal candidiasis is a common infection in women worldwide. Definitive laboratory procedures are of paramount importance to identify *Candida* isolates from suspected VVC cases to a species level to ensure appropriate and effective use of antifungal agents.

Key words: *Candida albicans*; Non-albicans species; Vulvovaginal Candidiasis; reproductive age; women; Haryana province

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INTRODUCTION

Vaginitis is one of the commonest reproductive tract infections and is characterized by vaginal discharge; vulvar itching/irritation, and malodor.[1] The abnormal vaginal discharge results from a variety of infectious and non-infectious causes. Infectious vaginal discharge can be caused by bacterial, viral, fungal or parasitic infections,

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the three most common causes being Bacterial Vaginosis (BV), Vulvovaginal Candidiasis (VVC), and Trichomoniasis. Non- infectious causes of abnormal vaginal discharge are many including an idiopathic condition known as desquamative inflammatory vaginitis (DIV), chemical irritants, vulvovaginitis associated with estrogen deficiency and other gynaecological conditions.

Candida albicans are part of the lower tract flora in 20-50% of healthy asymptomatic women. Vulvovaginal candidiasis is caused by the overgrowth of Candida species in the vagina and is characterized by itching, erythema and curd like vaginal discharge.[2] The changes in the composition and function of the vaginal microbiota may expose females to *Candida* infection. Vulvovaginal candidiasis is triggered by hormonal changes such as puberty, pregnancy, menopause, use of hormonal contraceptive or hormone replacement therapy have a 25% life time risk.[3]

Surveys suggest that about 75% of women develop vulvovaginal candidiasis (thrush or yeast infection) at least once in their lifetime.[4] VVC is most often caused by *Candida albicans*, however, other species of *Candida* such as *Candida glabrata, Candida parapsilosis*, and *Candida tropicalis* are emerging.[5] Diagnosis of vulvovaginal candidiasis is established by a combination of microscopic examination showing yeasts, hyphae, culture, or a combination of all from a vulval or vaginal swab in the presence of compatible clinical signs and symptoms.[6]

The estimated global burden of vulvovaginal candidiasis is high, and with the changing age structure of the global female population and prevalence of diseases that increase risk for vulvovaginal candidiasis, it is likely to increase. The current study was designed to study microbiological diagnosis and anti-fungal susceptibility testing of vulvovaginal candidiasis in symptomatic women of reproductive age group attending our tertiary care institute.

MATERIAL AND METHODS

The present study was a prospective observational study, conducted in Department of Microbiology, Maharishi Markandeshwar Institute of Medical Sciences and Research, Mullana. The study period was from May 2016 to October 2016 (6 months).

Vaginal swabs collected from patients with abnormal vaginal discharge, attending gynecology OPD for the same complaint.

The Inclusion Criteria were female patients in the reproductive age group (15 to 50 years), with the complaint of excessive vaginal discharge were considered for the study. The Exclusion Criteria was patients with bleeding per vagina, pregnant, menstruating females and those who received vaginal antibiotics (pessary) in the past 3weeks or refused to give consent were excluded.

Study was explained to patients and a written informed consent was taken from patients. Details of the patients such as age, symptoms, type of discharge were noted.

The samples were processed for direct examination of the *Candida* specieson malt yeast extract agar (MEA) media. For direct microscopic examination, a small portion of the specimen was mounted in 10% KOH and observed microscopically to see the presence or absence of budding cells and the sporulating structures. For selective isolation of a *Candida* spp., the samples were inoculated on four different media: Malt yeast extract agar, Candidchrom agar, Biggy agar, Cornmeal with tween 80 agar and incubated at 250 C, 370 C and 400 C for 24-48 hrs. The plates were examined for the presence of growth and sporulating structures.

Isolation was considered significant only if the fungal growth coincided with the inoculation streaks and same fungus was obtained. The purified isolates were identified on the basis of morphological characteristics in lactophenol cotton blue wet-mount microscopy (presence of budding). All the isolates were purified on MEA plates. Three different species of *Candida* were identified from the patients. Anti-fungal susceptibility testing (AFST) was done by Disc Diffusion Test in Mueller Hinton Agar (MHA).

Results of the culture and antimicrobial sensitivity testing were documented. Collected data was entered in Microsoft excel sheet and analysed. Statistical analysis was done by descriptive statistics using univariate analysis for calculation of distribution among groups in form of percentage.

RESULTS

During study period 300 patients satisfying inclusion and exclusion criteria underwent microbiological study of vaginal discharge sample to diagnose vulvovaginal candidiasis. Total 300 samples were tested, out of which 18.33% (55 samples) were tested positive for candidiasis. Positive samples were studied further. In present study 26-30 years age group (25%) was most common followed by age group 31-35 years (24%) as shown in Table 1.

Following characteristics of vaginal discharge were noted in present study. Most patients had curdy white (85%), moderate (60%), thick consistency (75%) discharge, for more than 15 days (65%) with malodour (78%) (Table 2).

Table 1: Age distribution

Age Group (in years)	No of patients (n=55)	Percentage
15-20	1	2
21-25	9	16
26-30	14	25
31-35	13	24
36-40	10	18
41-45	8	15

Table 2: Attributes of vaginal discharge

Attribute	No of patients (n=55)	Percentage
Colour		
White	8	15
White curdy	47	85
Quantity		
Scanty	9	16
Moderate	33	60
Copious	13	24
Consistency		
Thin	14	25
Thick	41	75
Duration		
≥15 days	19	35
<15 days	36	65
Malodour		
Present	43	78
Absent	12	22

Table 3: Candida species isolated from high vaginal swabs

Candida species	No. of isolates (%)
Candida albicans	36 (65)
Candida tropicalis	13 (24)
Candida glabrata	6 (11)

Out of 55 isolates, *Candida albicans* (65%) was most common followed by *Candida tropicalis* (24%) and *Candida glabrata* (11%) (Table 3).

Antifungal susceptibility was best for amphotericin B followed by clotrimazole, miconazole and least for fluconazole as noted in Table 4.

DISCUSSION

Abnormal vaginal discharge also predisposes women to significant morbidity in the form of pelvic inflammatory diseases, infertility, endometriosis, cuff cellulitis, urethral syndrome, pregnancy loss, preterm labor, increase susceptibility to sexually transmitted infections (STI), including HIV.[7,8]

Women with abnormal vaginal discharge feel "dirty" and suspicions about sexually transmitted infection acquired from their partner are almost universal. Male partners can develop penile irritation; consequent to vulvovaginal candidiasis. The severity of symptoms in women with vulvovaginal candidiasis varies from moderate to severe, but invariably affects quality of life and is associated with considerable stress.[9]

Numerous risk factors have been reported as being associated with VVC, such as pregnancy, use of broad spectrum antibiotics, uncontrolled diabetes mellitus, use of contraceptives and hormone replacement therapy, use of corticosteroids, cancer chemotherapy, organ transplantation, tight-fitting clothing, synthetic underwear, various dietary deficiencies or excesses, increase sexual activity, and vaginal douching.[4,10] Existing data pertaining to some of these factors on the risk of developing VVC are conflicting.[11]

In the present study, the prevalence was found to be 18.3%. Different studies from India have reported the prevalence of vaginal candidiasis as 21.31% by Nandan D et al,[12] 19% by Aring BJ et al[13] and 14 % by Rao RP.[14] In present study, age group 26-30 years and 31-35 years together comprise more (49%) number of patients.

In the study of Gandhi TN et al, most common age group was 26 to 35 years.[15] In another study conducted by Kavitha Y et al.[16] most commonly isolated species were *Candida albicans* 31 (43.66%) followed by *Candida tropicalis* 27(38.03%) and *Candida glabrata* 13(18.31%). Similar findings were noted in present study. Other Indian studies by Sasikala and Udayasri,[17] Rajeshwari PR[18] reported isolation of C. albicans as 46.1% and 54% respectively.

Deorukhkar et al,[19] has shown that Diabetes mellitus (DM) results in both increased rate of vaginal *Candida* colonization and infection with *Candida*. The occurrence of VVC among women with diabetes may be attributed to poorly controlled diabetes mellitus which causes increased glycogen levels and other metabolic alterations, which lower vaginal pH resulting in *Candida* colonization at a rate higher than that of vaginal dysbiosis.[20]

Vulvovaginal candidiasis is classified as either uncomplicated or complicated disease based on clinical presentation, host factors, microbiology, and response to therapy.[21] Most women with VVC suffer from uncomplicated vaginitis. This presents as sporadic cases of mild to moderate infections usually due to C. albicans, and these cases occur predominantly in healthy adult women who have no predisposing factors.

About 10-20% of women suffer from complicated VVC that is characterized by more severe attacks or are caused by NAC species with diagnostic and therapeutic implications.[22] Infections in pregnancy or associated with or other concurrent conditions, such as

Table 4: Antifungal susceptibility of candida species

Candida species	Clotrimazole (%)	Fluconazole (%)	Miconazole (%)	Amphotericin B (%)
Candida albicans	29 (81)	15 (42)	26 (72)	36 (100)
Candida tropicalis	8 (62)	4 (31)	6 (46)	13 (100)
Candida glabrata	2 (33)	2 (33)	3 (50)	6 (100)

immunosuppression and uncontrolled diabetes, are also categorized as complicated infections.[23] Traditionally, *C. albicans* has been regarded as the principal etiologic agent of VVC; however, *non-albicans Candida* (NAC) species have recently gained scientific and epidemiological interests as their prevalence is on the increase globally. NAC species are identified more often in complicated VVC than uncomplicated cases.

Most cases of NAC vaginitis are due to *C. glabrata, C. krusei, C. parapsilosis, C. tropicalis, C. dubliniensis,* with *C. glabrata* predominating.[19] Diabetes mellitus, older age, prior antifungal agents use, and low socioeconomic status are conditions that result in higher likelihood of non-albicans candidiasis. An important characteristic of NAC species is intrinsic resistance or low dose susceptibility to the azole antifungals, the first-line treatment, resulting in treatment failure.[24]

Vulvovaginal candidiasis is also classified as sporadic (acute) and recurrent based on the frequency of the infectious episodes. VVC can be recurrent or relapsing. Those who have had at least four episodes of VVC during a 12-month period are considered to have recurrent VVC (RVVC). Up to 10% of women of reproductive age group have this infection as a recurrent condition.[25]

Recurrent vulvovaginal candidiasis (RVVC) is part of the complicated vulvovaginal candidiasis. There are two forms of RVVC: primary RVVC which is idiopathic with unknown predisposing factors. This occurs in otherwise healthy, immunocompetent women, majority of who have no discernible precipitating or causative factors.[26] Secondary RVVC is the occurrence of frequent episodes of acute VVC because of certain predisposing factors such as hormone replacement therapy or diabetes mellitus.[27]

All these clinical entities are important while treating patients. Microbiological diagnosis & anti-fungal susceptibility testing is very important while treating women with RVCC, women with predisposing factors. Due to increasing resistance to routinely used antifungal agents like fluconazole and clotrimazole judicious use of antifungal agents is very important. Education regarding personal hygiene to couples is useful to prevent vulvovaginal candidiasis.

CONCLUSION

Vaginal candidiasis is a common infection in women worldwide. *C. albicans* is the most common etiology but there is an ongoing increase in the prevalence of NAC species in vaginal and indeed other candida infections due to increasing use of antifungal agents and susceptible patient population. Definitive laboratory procedures are of paramount importance to identify *Candida* isolates from suspected VVC cases to a species level to ensure appropriate and effective use of antifungal agents.

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G - Concept and design of the study, collection of data, prepared the first draft of manuscript; **HM -** Interpreted the results, revision of manuscript.

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