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ORIGINAL RESEARCH

Prevalence, Species Distribution and Virulence Factors of Candida Species Isolated from Cases of Vulvo Vaginal Candidiasis from a Tertiary Care Hospital

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ABSTRACT

Background: Candida species like Candida albicans and non candida albicans are the major cause of vulvo vaginal candiasis (VVC). These organisms produce an array of virulence factors which makes it to initiate the infection. The present study is undertaken to study the fungal causative agents, their species distribution and virulence factors from the Candida species isolated from the cases of VVC from a tertiary care hospital. **Methods:** All female patients in the reproductive age group (18-49 years) fulfilling the clinical criteria of are included in the study. Phenotypic characterisation was done by Gram stain morphology, KOH wet mount examination and culture on Sabouraud's dextrose medium. Species differentiation determined by growth on CHROM agar and sugar assimilation tests. Virulence factors like formation of biofilm, proteinase and phospholipase are tested by standard methods. **Result:** A total number of 116 (33.1%) subjects showed pure growth of Candida species by conventional standard methods. The most common species isolated by conventional methods was C.albicans (36.3%) followed by C. glabrata (24.1%), C. tropicalis (22.5%), C. krusei (10.3) and C.parapsilosis (7.7%). Among biofilm, proteinase and phospholipase producers, it was seen that C. albicans (46.5%, 48.1% & 41.8% respectively) was the commonest producer. Followed by C. glabrata, the commonest biofilm, proteinase & phospholipase producer was (23.2%, 23.4 & 25.4% respectively). **Conclusion:** Identification and species differentiation is essential for the management of cases with VVC. Virulence factors were produced in significant amount by the isolated strains of Candida species.

Key words: vulvo vaginal candiasis, Candida albicans, Non Albicans Candida, virulence factors.

INTRODUCTION

Infections of vulva and vagina are commonly encountered in gynaecological practice. These infections are predominant in the women of reproductive age group, and often clinically manifest as discharge from vagina. Many bacterial and fungal agents are responsible for these vulvovaginal infections of which candidiasis is the most common one. Vulvovaginal candidiasis (VCC) contributes to over one third of all the vaginal infections, and more than 70% of the women in reproductive age group present with VVC at least once during their lifetime. Moreover, it has been estimated that about 8% of the women experience recurrent VVC infections.[1] The most common and clinically relevant species of VVC is Candida albicans, recent studies suggested that there is a cumulative frequency of isolation of non albicans candida species like Candida glabrata, Candida krusei and Candida parapsilosis.[2] in the non albicans candida species, C glabrata is considered the most common[3,4,5]. The virulence of Candida species depends on various intrinsic factors including expression of adhesins, development of hyphae, biofilm formation and secretion of hydrolytic

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enzymes. Adhesins are biomolecules which help in adherence of the fungus to the host and body of other microorganisms. Speciation of Candida isolates is useful not only for epidemiological purpose but also helps to recognize strains virtually resistant to some of the antifungal agents[6,7]. Formation of biofilm is related with high level of antifungal resistance. The present study aims at isolation, characterisation, species differentiation and virulence factors of Candida species isolated from the VVC cases at a tertiary care hospital.

MATERIALS AND METHODS

The study was conducted in the Department of Microbiology, at a tertiary care hospital of central India. A prospective, non-randomized, observational study on patients visiting the hospital. A total of 350 samples were collected from the patients attending the obstetrics and gynecology Department. The informed consent was taken from the study subjects and three high vaginal swabs per patient was collected and transported to the laboratory. Potassium hydroxide wet mount preparation and gram staining were done using two swabs and the isolation of the organism is done on Sabouraud's dextrose agar was done by using the third swab. The inoculated plates were incubated at 370C for 48 hours. Species identification done by using standard biochemical tests like germ tube formation, chlamydospore formation, sugar assimilation tests, growth on CHROM agar, tetrazolium reduction medium and carbon and nitrogen assimilation tests. Virulence determinants like ability to form biofilm, proteinase production, phospholipase production was done with standard procedures

RESULT

A total no of 350 samples were included in the study, out of these 116 (33.14%) samples showed pure growth of Candida species. The isolation of non albicans Candida (NAC) was higher (62.9%) than Candida albicans (36.2%) among the subjects studied. 82.75% of married ladies have found positive for vulvovaginal in comparison with unmarried girls in the study group. Culture positivity was seen in age group of 21- 30 years (37.8%), followed by 31-40 years (32%) and 41-50 (21.2%). The higher positivity in age group 21-30 years was statistically significant ((p value - 0.014). in comparison to other age groups. Candida positivity was found to be higher among pregnant (42.9%) as compared to that among non-pregnant (27.1%) subjects included in the study. Among the patients presenting with one or more risk factors studied, 33.14% were positive for Candida. Candida positivity in patients having history of diabetes mellitus together with bad obstetric history was 100%. This was followed by that in patients who were pregnant. (45%), diabetes mellitus alone (33.3%), those taking oral contraceptives (30%) and multigravida (25.4%) were other risk factors for infections. The various Candida species isolated on the basis of colony characteristics, morphology on corn meal agar, germ tube & chlamydospore formation, sugar fermentation & sugar assimilation reactions were C. albicans (36.3%) is the most common isolated species followed by C. glabrata (24.1%), C. tropicalis (22.5%) and C. krusei 1(0.3%). Total 116 isolates were showed the presence of one or more of the three virulence factors studied such as formation of Biofilm, production of proteinase & production of phospholipase production. The present study observed that the virulence factor of phospholipase was shown by the most strains (86.2%) followed by biofilm formation (75.8%) and proteinase production (70.6%). The most common isolated strain, C.albicans, showed virulence factors like phospholipase (95.2%), followed by biofilm formation (59.7%) and proteinase production (80.9%).The isolates of non candida albicans species, mostly produced phospholipase (80%), followed by biofilm production (70.2%) and proteinase production (64.8%).

DISCUSSION

Vulvovaginal candidiasis is the most common infection of women in lower genital tract in the reproductive age group, most of the women will have the infection at least once in their life. [12] In our study the prevalence of the infection of symptomatic women was 33.14% this study correlates very closely with the study done by Libya (36.1%) 1 and Kalia N et al. (31%).[13,14] Studies have reported the low prevalence rate of VVC from K wawukume EY et al.[15] 25%, Mirza NB et al. [16] 24% and Otero L et al.[17] 18.5%. The prevalence rate is very higher as comparison to our study done by EA Ugwa (84.5%) in North- West Nigeria.[18] In the present study the frequency of vulvovaginal candidiasis was higher in the age of 21-30 (37.8%). In age group of 21-30 the high prevalence rate was also reported by Sehgal et a1.[19] (73.3%), Onuorah Samuel et a1.[20] (66.7%) and, Toua V et al.[21] (55.4%). Study done by Emeribe et al.[22] and Puri et al.[23] shown that maximum vulvovaginal candidiasis infection was found in between the age group of 21–40 years which was the most sexually active age group. EA Ugwa [24] reported highest prevalence in 26-35 years age group (53%).In our study we observed that 42.9% of vulvovaginal candidiasis infection was confirmed in the pregnant women as compared to other risk factors, though this difference was statistically significant. Ragunathan L et al. [25] (45.3%), Neerja J et al.[26] (42%) and Bankar et at [27] (48.97%) described that pregnancy is the major predisposing risk factor of vulvovaginal candidiasis.

The study done by Sobel et al [28] and Okungbova et al. [29] pregnancy was the most common associated condition, the reason being attributed to elevated steroid hormones in pregnancy which makes the vaginal mucosa acidic, predisposing it to vaginal

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infection. In another study done by Ragunathan L at al.[30], 55% females who reported to VVC infection had pregnancy. This most likely happened because increased level of hormones during pregnancy, which is the energy source for *Candida* growth.

Present study showed a significant variation in the distribution of Candida species. The most predominant species was found to be C. albicans (36.3%), followed by C. glabrata (24.1%), C. tropicalis (22.5%), C. krusei (10.3%), and C. parapsilosis (7.7%), respectively our findings are similar to the previously reported data by Sajjan et al[31]. Zarei-Mahmoudabadi et al.[32] and Chakrabarti et al.[33] reported the prevalence rate (39% and 25%) of C. albicans.

In present study *C. albicans* was the commonest species isolated 36.3%. Previous report from Egyp El-sayed H et al. (86.6%) [34], Alfouzan W et al Kuwait (73.9%) [35] Al- mamari A et alYemen (65.9%) [36] & Al-Hedaithy S et al Saudi Arabia (59%) [37] have also reported the highest isolation rate of *C. albicans in* VVC. Worldwide, rates of the isolation of *C. albicans* in cases of VVC ranges between 47% and 89% in studies from Darce Bello M et al. Nicaragua[38], Holland J et al. and Pirotta M et al. Australia [39,40], Gultekin B et al Turkey [41] Pakshir K et al Iran, [42] ،and Babin D et al India [43].

In present study the isolates of *NAC* were higher (62.9%) compare to the isolates of *C. albicans* 36.2%. The highest isolation of *NAC* over *C.albicans* has also been reported by Deepa Babin et a1.[44] (64.5% vs. 35.5%) Kikani B et a1.[45] (55.6% vs. 44.4%), and Namrata et a1 [46] (53% vs. 47%).In present study total of 116 isolates presented the presence of one or more of the virulence factors (biofilm formation, proteinase and phospholipase production) studied. The presence of all of these virulence factors were seen more in *C. albicans* as compared *to non albicans Candida.* In present study, production of proteinase was seen more among *C. albicans* (80.9%) as compared to *NAC* (64.8%). Several studied has been reported proteinases are produced higher rates by *C. albicans,* as compared to *NAC.* Jasim ST et al. [47] reported that maximum proteinase production was observed in *C. albicans* isolates (79.5%), as compared to *non-C. albicans* candida species (63.63%).

The study done by Costa et a1. [48] 88.1% of *Candida albicans* and 69.8% of *non- albicans Candida* isolates produced proteinase. The proteinase-producing capacity *of non albicans candida* (50.45%) was slighter than that of *C. albicans* (67.34%). According to M. Vinitha et a1. [49] proteinase detected activity is 74.56% of Candida species isolated from the blood samples. Another study done by Dan et al.[50] showed the higher proteinase production (85.0%) in Candida isolates; this rate reached 100% in non-C. albicans species. [51] Also similar result given by Tsang et al. (2007) [52], maximum proteinase production was reported in C. albicans (82.1%), followed by non C. albicans species (80%) [53] Some another study also reported by Sachin et al. (2012), proteinase production was found in 65 (59.1%) Candida isolates.[54] In the present study the *NAC* isolates have maximum proteinase positivity was seen in *C. glabarata (23.45%), tropicalis* (18.51%), and other species. Vivek K S et al.[55] reported that proteinase activity is 61.4% of their isolates, and maximum proteinase activity in *C. albicans* they detected followed by *C. glabrata, C.tropicalis and C.krusei.* They are suggested that the secreted proteinases are responsible for the adhesion & tissue invasion.S Katy et a1. [56] reported that proteinase activity is 33.3 % in *C. tropicalis* and 20% in *C. glabrata,* followed by 15.3% of *C. parapsilopsis,* retrieve from vaginal secretions. This study closely related to our study especially of *C. glabrata.*In this study, the phospholipase activity was more associated with *C. albicans* (95.2%) as compared to *NAG* (80%) (Table: 13). Costa et a1.[57] reported that production of phospholipase is 55.9% in *C. albicans* and only in 37.7% *non-albicans Candida.* M.Vinitha et al.[58] reported that phospholipase activity was detected in 14% isolates, which included 46.93% of *C. albicans* and 42% *NAC.* S Katy et a1.[59] demonstrated phospholipase activity in 8.9% isolates vaginal secretions and maximum production was seen in *C. albicans* 42.8%) isolates. Another study conducted by Samaranayake et al.[60] 80% isolates of *C. albicans* recovered from HIV patients were phospholipase- positive. Biofilms are universal, complex, interdependent communities of surface-associated microorganisms, enclosed in an exopolysaccharide matrix occurring on any surface, including medical devices.[61] The pathogenicity of Candida species is associated with its ability to form Biofilm and is an essential virulence determinant during candidiasis.[62] the present study showed biofilm production to be 85.7% among C. albicans spp. and 70.2% among non- albicans candida. This result is similar to those reported by Kumar et al.[63] However, Kuhn et al.[64] showed that C. albicans produces quantitatively more biofilms than other Candida species.

CONCLUSION

In the reproductive age category of women, vulvovaginal candidiasis is the most prevalent infection. A number of predisposing factors, including HIV, diabetes mellitus, IUCD usage, antibiotics, and immunosuppressive medication use, also contribute to vulvovaginal candidiasis. The most prevalent species was C. albicans, followed by C. glabrata and C. tropicalis, as determined by the culture method of species identification. It is important to exercise caution when specifying candida using CHROM agar, particularly when identifying non-albicans candida. The present study concluded with the idea of various candida species isolated in the cases with VVC and their species distribution. Identification of species and the determination of virulence factors are essential

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components of the laboratory confirmation and study of the progression of VVC, which are mainly produced by Candida albicans and non-albicans candida.

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