Clinico-Mycologicalprofile of Vaginal Candidiasis in A Tertiary Care Hospital

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ABSTRACT
Candida species are opportunistic yeasts affecting the genitourinary tracts. A study was carried out to determine the frequency of vaginal candidiasis and the susceptibility profile of the Candida species to antifungal agents. The study was carried out prospectively in Tirunelveli Medical College Hospital, Tamil Nadu during August and September, 2013. 50 vaginal swabs were collected from sexually active women in the age group of 18-45 and cultured, out of which 12(24.00%) were positive for Candida species. They were tested for antifungal susceptibility by disk diffusion method. C. parapsilosis (58.33%) was the predominant species isolated followed by C. albicans (25.00%) and C. glabrata (16.67%). The highest frequency of vaginal candidiasis was seen in the age group of (26-35). Usage of antibiotics was the most frequently implicated risk factor (41.67%). Susceptibility testing revealed that 66.67% of C.albicans, 50% of C. glabrata and 57.14% of C. parapsilosis were sensitive to Itraconazole, 66.67% of C. albicans were sensitive to Fluconazole. 57.14% of C. parapsilosis were sensitive to Clotrimazole and 71.43% were sensitive to Miconazole.In conclusion, an increase in non-albicans Candida infections and the emergence of Azole resistant C. albicans and non-albicans species which emphasizes the need for species identification and antifungal susceptibility as a part of laboratory diagnosis of vaginal candidiasis.

Introduction:

Vaginal candidiasis is one of the most common vaginal infections in women, in the fertile period, and also the most frequent and most important fungal disease. Until recently, the problem of vaginal candidiasis was often ignored or treated as an insignificant problem among the female population.[1] In addition, many psychological and emotional stress related problems are associated with vaginitis such as; reduced immunity, prolonged antibiotic therapy, use of contraceptives, malnutrition, pregnancy, diabetes, obesity, tissue transplant, use of immunosuppressive agents, neutropenia etc.[2]

Presence of indwelling central venous catheters and prior haemodialysis has also been identified as a risk factor. Sexual intercourse with an infected person is also a mode of spread of genital candidiasis [3].There is a balance between Candida, normal bacterial flora, and immune defence mechanisms. When this balance is disturbed, colonization is replaced by infection. It is possible that there are multiple mechanisms by which Candida can cause cell damage and lead to direct invasion of hyphae in epithelial tissues. During vaginal candidiasis, vagina is in the normal pH range (pH 4- 4.5), as opposed to mixed infections (Bacterial, Trichomonas), where pH levels rises.[4]

Numerous studies around the world showed that Candida albicans is responsible for the largest number of symptomatic episodes of vaginal candidiasis. Percentage of non-albicans was high in the recent decades and varied from 85 to 90%. Non-albicans species are most commonly represented by C. tropicalis, C. Parapsilosis, C.glabrata, and C. krusei. [5] Accurate species identification is important for the treatment of the Candida infections, as the non-albicans species of Candida continue to be increasingly documented. Hence, the objective of the study was to document the frequency of vulvovaginal candidiasis, speciation of the isolates of Candida and determine the susceptibility profile of the Candida species to antifungal agents.

Material and Methods

This prospective study was carried out in Obstetrics and Gynaecology Department, Tirunelveli Medical College Hospital, TamilNadu, India from May to October 2013. The study protocol was approved by the ethical committee of the institution. The patients gave written informed consent to participate in the study.

Inclusion criteria:

Married, pregnant and sexually active women between 18-45 yrs of age who presented with self-reported symptoms of vaginal discharge, genital burning or burning during micturition during the study period were included.

Exclusion criteria:

Women of age group below 18 and above 45 yrs, patients with cervical malignancies, women in menopause were not included in the study.

Specimen collection:

Vaginal specimens were collected using cotton swabs after the speculum was inserted into vagina to separate the vaginal walls and wiping away the excess cervical mucus. They were then properly labelled and transported to the laboratory.

Laboratory procedure:

Samples were inoculated on SDA and incubated at 37 degree Celsius. Plates were examined after 48 hours incubation. Smear was prepared and stained by Gram's Method for microscopic examination. Candida species were identified based on colony morphology, germ tube testing and pigmentation on CHROM agar.[6,7]All the isolates were subjected for antifungal susceptibility testing for Ketoconazole, Clotrimazole, Nystatin, Miconazole, Fluconazole, Itraconazole, Voriconazole by Disc diffusion method with M44-A Clinical Laboratory Standards (CLSI) guidelines.[8]

Results:

The present study included 50 female patients who presented with genital manifestations, suspicious of candidial infection. The study revealed an overall isolation rate of 24.00% (12/50) candidiasis from vaginal swabs.

All the 12 isolates of Candida species were processed for speciation and antifungal sensitivity testing. In the present study we were able to characterize three species of Candida. C.parapsilosis (58.33%) was the predominant species isolated followed by a higher incidence of C.albicans (25.00%), C. glabrata (16.67%). The overall rate of Non-albicans isolates were 75.00% showed its increased prevalence. The highest prevalence of vaginal candidiasis (41.67%) was seen in the age group of (26-35).(Table 1)

The predisposing factors of vaginal candidiasis from positive isolates were analysed in which use of antibiotics (41.67%) has been the most frequently associated risk factor followed by pregnancy (25%), intrauterine devices (16.67%), and diabetes mellitus (16.67%). (Table 2)

Antifungal susceptibility testing was performed by disk diffusion method and it showed that all the 12 isolated Candida species were 100% susceptible to Ketoconazole, Nystatin and Voriconazole. Whereas 66.67% of C.albicans, 50% of C. glabrata and 57.14% of C. parapsilosis were sensitive to Itraconazole, 66.67% of C. albicans were sensitive to Fluconazole while C. glabrata and C. parapsilosis were 100% sensitive to Fluconazole. Of the 7 isolates of C. parapsilosis, 57.14% were sensitive to Clotrimazole and 71.43% were sensitive to miconazole. However C. albicans and C. glabrata were 100% sensitive to Clotrimazole and Miconazole. The highest resistance of 50% was seen with C.glabrata to Itraconazole. (Table 3)

Discussion:
Vaginal candidiasis is an extremely common infection in 60-70% women during their reproductive age at least once in their lives [9, 10]. The present study had revealed the highest incidence rate of candidiasis 24.00% (12/50), which is similar to the study of Akortha et al [11] Saldanha et al [12] and Srujana et al [13].

The present study showed the increase in frequency of non-albicans species as potential causes of vaginal candidiasis. Most frequently isolated non albicans in our study have been C.parapsilosis (58.33%) and C. glabrata (16.67%). Our findings have also showed that 25.00% of C .albicans was identified from 12 positive cases. Thus the overall prevalence of non albicans species in our study was (75.00%) which is more than that of C .albicans (25.00%). It is important to emphasize that in the past three decades there has been an increasing percentage of infections caused by non-albicans species of Candida, particularly, C. tropicalis, C.glabrata, C. parpsilopsis and C. krusei and its resistance to conventional therapy. C. glabrata was the most common species among the vaginal isolates (56, 50.4%) followed by C. albicans (39, 35.1%) in the study of Srujana et al [13].

We speculate this increasing detection of non-albicans species are probably related to the widespread and inappropriate use of anti fungal treatments (self medication, topical use, long-term treatments and repeated candidial episodes). Hence, the reliable and rapid identification method of Candida species is a fundamental goal of microbiology laboratories.

The highest frequency of vaginal candidiasis (41.67%) was observed in the age group of 26- 35 years, followed by the age group of 18-25. Lower frequency results (25.00%) were obtained in the age group of 36-45 in our study. The study of Ako et al [14] supports that vaginal candidiasis occurs most frequently in the age group 20-25. And the reports of Sehgal [15] also showed the age group 21-30 years had the highest incidence of vaginal candidiasis. So women of childbearing age groups are more vulnerable to vaginal candidiasis.

The predisposing factors of vaginal candidiasis from positive isolates were analysed in which use of antibiotics (41.67%) has been the most frequently associated risk factor followed by pregnancy (25%), intrauterine devices (16.67%), diabetes mellitus (16.67%).

History of antibiotics, corticosteroids usage (41.67%) was the most common predisposing factor associated with vaginal candidiasis in our study. Antibiotic usage will cause the elimination and alteration of normal bacterial flora and allows the colonization of Candida. Our findings show agreement with studies of Jinping Xu [16] that use of antibiotics favours candidiasis.

Pregnancy (25.00%) was the second most common predisposing factor associated with vaginal candidiasis in our study. Our findings show agreement with studies of Sobel [9] and Okungbova et al [17], Ahmet et al [18] that high level of reproductive hormones and increase glycogen content of vagina favours candidiasis in pregnancy.

The other major risk factor was the use of Intrauterine devices. In our study, 16.67% of patients had developed vaginal candidiasis due to the usage of vaginal candidiasis. So pregnancy, use of chemotherapeutic agents and IUDs had been identified as risk factors associated with vaginal candidiasis in our study. These risk factors associated in our study correlated well with the studies of Mirela et al [19] and Lundstrom et al [20].

In vitro antifungal susceptibility testing is becoming important because of the emergence of new non albicans strains and the increased inherent and acquired resistance to azoles and Amphotericin-B. Antifungal susceptibility testing in our study revealed that all of the Candida albicans isolates tested was susceptible to Ketoconozole, Voriconazole, Clotrimazole, Nystatin and Miconazole. For Fluconazole and Itraconazole, the overall susceptibility rate for C. albicans was 66.67%. C. parapsilosis isolates were resistant to Itraconazole (42.86%), Clotriconazole (42.86%) and Miconazole (28.57%). However they showed a 100% susceptibility to Fluconazole, Voriconazole, Nystatin and Ketoconazole. C. glabrata showed 50% resistance to itraconazole but showed 100% sensitivity to all other drugs. Our findings correlate with the study of Saldanha et al [12] and Noake et al [21] which showed Candida species show 50% resistance to azoles.

Most non-albicans Candida species in our study had higher azole resistance and infections they cause are often difficult to treat. C. parapsilosis showed higher resistance to Miconazole, Clotrimazole and Itraconazole in our study. A similar study by Sobel [9] showed higher resistance. One of the possible explanations for more frequent isolation of non-albicans species from vulvovaginitis patients may be the increased use of topical azole agents. So the present study emphasizes the need for testing the antifungal susceptibility tests for the Candida isolates to control the spread of new resistant strains in the future.

Conclusion:
The epidemiological profile of vaginal candidiasis varies from country to country as well as within the country depending upon so many factors like socioeconomic and health factors. Whereas in India there is an increase in infections with non-albicans Candida spp and the emergence of azole resistant C. albicans and non albicans species also specify the need of species identification and antifungal susceptibility as a part of the laboratory diagnosis of vaginal candidiasis. The emergence of itraconazole resistance in non albicans Candida may caution against its use as a prophylactic agent in hospitals. Effective antifungal treatment is an important criterion in treating the candidial infections. Therefore, screening programme is essential to monitor the antimicrobial resistance. The epidemiological status of candidiasis plays an important role in the control strategies. This study also provides the baseline information on the prevalence and antifungal susceptibility pattern of Candida isolates in our region.

Table 1: Age wise distribution of patients from which Candida species isolated

Age wise	C. albicans	C. glabrata	C. parapsilosis	Percentage age wise
18-25	2	1	1	33.33
26-35	1	0	4	41.67
36-45	0	1	2	25.00
Total	3	2	7	24.00
Total Percentage	25.00	16.67	58.33	Percentage of Non-albicans spp 75.00

Table 2: Predisposing factors for Vaginal Candidiasis

Predisposing factors	Number of patients	Percentage of isolation
Pregnancy	3	25.00
History of Antibiotics	5	41.67
Intrauterine devices	2	16.67
Diabetes Mellitus	2	16.67

Table 3: Antifungal susceptibility pattern by disk diffusion method

Antifungal agents	No. (n)	Fluconazole		Itraconazole		Voriconazole		Clotriconazole		Nystatin		Miconazole		Ketoconazole		
		S%	R%	S%	R%	S%	R%	S%	R%	S%	R%	S%	R%	S%	R%	
C. albicans	3	66.67	33.33	66.67	33.33	100	0	100	0	100	0	100	0	100	0	5%
C. glabrata	2	100	0	50.00	50.00	100	0	100	0	100	0	100	0	100	0	
C. parapsilosis	7	100	0	57.14	42.86	100	0	57.14	42.86	100	0	71.43	28.57	100	0	

S = Susceptible, R = Resistant

REFERENCE [1] Sobel JD, Faro S, Force RW.Vulvovaginal candidiasis: Epidemiologic diagnostic and therapeutic considerations, Am J Obstet Gynecol 1998; 178:203-211. | [2] I. Francisca, Okungbowa1, S. Omoanghe, The distribution frequency of Candida species in the genitourinary tract among symptomatic individuals in Nigerian cities, Rev Iberoam Micol 2003;20:60-63. | [3] Tatfeng YM., Agba MI., Nwobu GO., Candida albicans in Urinary Tract or in Seminal Sac, Online J Health Allied Scs.4;2003:5. | [4] Anderson JL, B.Cundiff, Schnars, Hypha formation in the white-opaque transition of Candida albicans, Infect Immun. 1989; 57:458–467. | [5] Mahmoudi Rad M., Zafarghandi S., Abbasabadi B., The epidemiology of Candida species associated with vulvovaginal candidiasis in an Iranian patient population, Euro J Obs Gyn Rep Biol 2011;155:199–203. | [6] Kwon Chung KJ, Bennett JE, Medical Mycology, Lea & Fibiger, Philadelphia.1992. | [7] Chander J., Candidiasis: A text book of Medical Mycology, Mehta Publishers, New Delhi, 2009:266-90. | [8] Wayne PA., Method for Antifungal Disk Diffusion Susceptibility Testing of Yeasts, CLSI M44-A.2004. | [9] Sobel JD. Vulvovaginal candidiasis, Lancet 2007; 369:1961-71. | [10]Jacqueline, Achkar, CF Bettina, Candida Infections of the Genitourinary Tract, Clin Microbiol Rev Apr 2010:253–273. | [11] Akortha. EE, Nwaugo VO, Chikwe NO. Antifungal resistance among Candida species from patients with genitourinary tract infection isolated in Benin City, Edo state, Nigeria, Afr J Microbiol Res 2009;3(11):694-699. | [12] Saldanha Dominic RM., Shivanand D., Species Identification of Candida Isolates in Various Clinical Specimens with Their Antifungal Susceptibility Patterns, Journal of Clinical and Diagnostic Research 2011; 5(6):1177-1181. | [13]Srujana Mohanty, Prevalence & susceptibility to fluconazole of Candida species causing vulvovaginitis. Ind J Med Res 2007; 126(9):3. | [14] Ako-Nai AK., Kassim, Adeniran MO. Study of urinary tract infections at Ile-Ife, Nigeria. East African Medical Journal 1993; 70:10-14. | [15]Sehgal SC, Epidemiology of male urethritis in Nigeria, J Trop Med Hyg 1990;93:151. | [16]Jinping Xu, MD, MS, Kendra Schwartz, Monina Bartoces, Joseph Monsur BS, Richard.K. Severson, Jack D. Sobel. Effect of Antibiotics on Vulvovaginal Candidiasis: A Metro Net Study J Am Board Fam Med. 2008; 21(4): 261-268. | [17] Okungbowa FI, Isikhuemhen OS, Dede AP.The distribution frequency of Candida species in the genitourinary tract among symptomatic individuals in Nigerian cities, Revista Iberoamerican de Micologia 2003;20:60-63. | [18]Baris A, Guzel, Ilkit M.An Evaluation of Risk Factors in Pregnant Women with Candida Vaginitis and the Diagnostic Value of Simultaneous Vaginal and Rectal Sampling, Mycopathologia. 2011; 172:25-36. | [19]Mirela Babi, Candida albicans and non albicans species as etiological agent of vaginitis in pregnant women, Bosnian Jourl of Basic Med Sciences 2010; 10(1):90-97. | [20] Lundstrom T, Sobel J. "Nosocomial candiduria" a review, CID 2001; 32:1602-1607. | [21] Noake T, Antifungal susceptibility of Candida species using the Clinical and Laboratory Standards Institute disk diffusion and broth micro dilution methods 2007; 19(3)::283-7.