European Journal of Obstetrics & Gynecology and Reproductive Biology 144 (2009) 68–71

Contents lists available at ScienceDirect

European Journal of Obstetrics & Gynecology and Reproductive Biology

journal homepage: www.elsevier.com/locate/ejogrb

Prevalence of *Candida* species and potential risk factors for vulvovaginal candidiasis in Aligarh, India

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ARTICLE INFO

Article history:
Received 27 April 2008
Received in revised form 6 December 2008
Accepted 22 December 2008

Keywords:
Vulvovaginal candidiasis
Candida species
Direct microscopy
Signs/symptoms

ABSTRACT

Objectives: The objectives were to determine the frequency of *Candida* species in women of different age groups as well as to suggest the criteria for the diagnosis of vulvovaginal candidiasis (VVC).
Study design: A prospective study of vulvovaginal candidiasis was carried out using laboratory diagnosis, with the estimation of vaginal pH and the direct microscopic and biochemical examination of vaginal discharge/secretions. Vaginal cultures for *Candida* species were collected from 1050 women with vulvovaginal symptoms.
Results: Out of 1050 women, 215 (20.47%) were positive for *Candida* species. Of 215 women, 172 (80%) had pH within the normal range and 167 (77.67%) were showing yeast cells and mycelia on direct microscopic examination. *Candida albicans* accounted for 46.9% of cases, *Candida glabrata* 36.7%, *Candida parapsilosis* 10.2%, *Candida tropicalis* 2.8%, *Candida krusei* 1.4%, and *Candida kiefer* 1.9%. The frequency of culture positivity was related to pregnancy ($P < 0.001$), an increase in parity ($P < 0.001$), and use of oral contraceptives ($P < 0.001$) and antibiotics ($P < 0.001$). The most common signs and symptoms in 215 women with positive cultures were pruritus with or without vaginal discharge and vaginal erythema.
Conclusion: Our study suggests that vulvovaginal candidiasis can only be diagnosed by using clinical criteria in correlation with vulvovaginal symptoms and *Candida* cultures.

1. Introduction

Every year almost 10 million women visit physicians with the common gynecological disorder, vaginitis [1]. Most of the women who attend health care clinics have the common problem of vulvovaginal candidiasis (VVC). Nearly 75% of all women have fungal vulvovaginitis at least once or more in their lives. Moreover, nearly 40–50% women may have a second episode of vulvovaginal candidiasis in their life, whereas 5% of women have reported having recurrent vulvovaginal candidiasis (RVC). It has also been reported previously that 75% of women are affected by VVC during their child-bearing years [2].

The most common clinical manifestations of vulvovaginal candidiasis are pruritus, hyperemia, vaginal discomfort and leucorrhea, burning, soreness, abnormal vaginal discharge, dyspareunia, and vaginal and vulvar erythema, which may cause problems in marital and sexual relations. The most common predisposing host factors are uncontrolled diabetes mellitus, immunosuppression, pregnancy, and hormone replacement therapy [3]. In addition to the other factors, immunity due to the local defense mechanism of the vaginal compartment and actual hormonal status are potential candidates for pathogenesis [4].

The rate of colonization with *Candida* and symptomatic vaginitis is higher during pregnancy. Moreover, the use of contraceptives has been widely accepted as a potential risk factor for *Candida* colonization [5]. In a study in which vulvovaginal candidiasis was self-diagnosed, more than half of the patients did not have yeast confirmed as a causative organism [6]. Most of the patients fail to respond to antifungal therapy, which is mainly due to an incorrect diagnosis [7]. The majority of true cases are caused mainly by *Candida albicans*. The mechanisms of defense in the host against VVC remain controversial [8]. The most common defense mechanism in the vagina against *C. albicans* is the innate immune response. *C. albicans* is a part of the normal vaginal flora in about 20–30% of women of childbearing age.

In view of the above background information, this study was undertaken to determine the frequency of *Candida* species in women of different age groups and to suggest criteria for the diagnosis of vulvovaginal candidiasis (VVC).

2. Materials and methods

One thousand and fifty women aged 15–60 years attending the obstetrics and gynecology outpatient department (OPD) with

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doi:10.1016/j.ejogrb.2008.12.020

complaints of vaginal discharge and/or vaginal itching and irritation were studied. After recording their personal medical symptoms and reproductive history, vaginal swabs were collected with the help of sterile transportable cotton swabs provided by Hi-Media, followed by microscopic examination. Vaginal samples were collected from the outer third of the vaginal wall with the help of a dry sterile cotton-tipped swab for pH determination. Next, the pH level of the vagina of all of the women was determined using pH indicator paper (Macherey-Nagel, Duren, Germany); the pH indicator range was 3.8–9. Vaginal discharge and secretions of women were collected from the upper part of the posterior vaginal fornix and the lateral vaginal wall using sterile cotton swabs (Hi-Media, Mumbai). Then, the smear was prepared from these samples and put onto glass slides. Microscopic examination of each patient was carried out to detect the presence of yeasts by Gram staining. Further microscopic examination was performed using saline and 10% KOH preparations and subjected to growth on Sabouraud Dextrose Agar (SDA) medium [9]. Presumptive species level identification was carried out using Hi-Chrome Candida agar (Hi-Media, Mumbai). Hi-Chrome Candida agar is a chromogenic medium with which a presumptive identification of *C. albicans*, *Candida glabrata*, *Candida tropicalis*, *Candida krusei*, and *Candida parapsilosis* can be made on the basis of the morphology and colors of the colonies [10]. Further species level identifications were made by germ tube production at 3 h incubation in serum at 35 °C, and the morphology of corn meal agar (CMA), sugar fermentation, and sugar assimilation tests were also performed [11]. Further confirmation was obtained using biochemical kits provided by Hi-Media, Mumbai. Data were statistically analyzed using the Chi-squared test. Ethics approval was received from the practitioners and patients attending the gynecology OPD, JNMC, Aligarh, UP, India.

3. Results

Of 1050 women, 215 (20.47%) tested positive for *Candida*. Of these 215 women, 172 (80%) had normal vaginal pH (4.0–5.0), whereas the remaining 43 (20%) had a pH value above 5. Our microscopic study for yeast cells and pseudohyphae was found to be positive in 167 out of 215 women with positive culture (77.67%). The ages of the women studied fell within the range 15–60 years. The present study showed that the women of the 21–25 age group had the highest frequency of *Candida*-positive cultures followed by the 26–30 age group, whereas the women aged above 46 years showed the lowest frequency of *Candida*-positive samples (Table 1).

A total of six species of *Candida* were isolated from 215 women. *C. albicans* had the highest frequency (46.9%) of the six *Candida* species. The percentage of non-albicans species (53.04%) was higher than that of albicans species (46.9%). Of the non-albicans species, *C. glabrata* was predominantly isolated (36.7%), whereas *C. krusei* was found to be the least prevalent candidate (1.4%). The percentages of *C. parapsilosis*, *C. tropicalis*, and *Candida kiefer* (10.2%, 2.8%, and 1.9%) are given in Table 1. The study of *C. albicans* distribution among different age groups revealed that women of the 21–25 age group had a higher frequency of *C. albicans*, followed by the 26–30 and 41–45 age groups. *C. glabrata* and *C. tropicalis* had the highest frequency among women of the 46–50 age group, whereas *C. parapsilosis* was found to be the most prevalent in the 36–40 age group. *C. krusei*, *C. tropicalis*, and *C. kiefer* were the most frequently found in women of the 15–20, 46–50, and 55–60 age groups, respectively (Table 1).

Of the 215 women with positive culture, 172 (80%) presented with complaints of pruritus with or without vaginal discharge and 43 (20%) with vaginal discharge only. The discharge varied from watery to homogeneously thick, with negligible or no smell (Table 2).

The potential risk factors for vulvovaginal candidiasis are evaluated in Table 3. Vaginal erythema was more common in women with positive cultures than in those with negative cultures ($P < 0.001$). The incidence of VVC was more common in pregnant women than in non-pregnant women ($P < 0.001$). There was a high incidence of VVC in women with parity of P0 and P1 compared with women with parity of >P2 ($P < 0.001$). A statistically significant difference was observed in the incidence of VVC between women who used oral contraceptives and women who did not ($P < 0.001$). Moreover, women using antibiotics were found to be more susceptible to VVC than non-users of antibiotics ($P < 0.001$). A continuous increase in the incidence of VVC was observed in women above the age of 30 years. The incidence of VVC was found to be more frequent among women with unsatisfactory genital hygiene than in those with satisfactory genital hygiene ($P < 0.001$). Our study also revealed a statistically insignificant difference between literate and illiterate women with regard to the occurrence of VVC ($P = 0.8$).

4. Discussion

For the diagnosis of VVC, vaginal culture is the most sensitive and accurate method of diagnosis compared with other methods [12]. Moreover, pH and microscopic studies of vaginal secretions could also be performed. In this study, 20.47% women were found to have VVC, which has also been reported in earlier studies [13].

Vaginal pH estimation is a simple and economical test; it has been widely used in cases of suspected VVC [14]. Previous studies have shown that in VVC, the pH of the vagina remains within the normal range [7,9,15]. Our study shows normal vaginal pH in 80% of patients with positive *Candida* cultures, whereas 20% of cultures show pH values >5 and this is probably due to mixed infection. The mean vaginal pH was higher in women with *C. glabrata* infection

Table 1
Species distribution of *Candida* in women of different age groups.

Age (years)	*Candida albicans* No. (%)	*Candida glabrata* No. (%)	*Candida parapsilosis* No. (%)	*Candida tropicalis* No. (%)	*Candida krusei* No. (%)	*Candida kiefer* No. (%)	Women in this age group	
							Positive culture No. (%)	Negative culture No. (%)
15–20	2 (25)	2 (25)	1 (12.5)	1 (12.5)	1 (12.5)	1 (12.5)	8 (3.7)	44 (5.3)
21–25	47 (50.5)	32 (34.4)	10 (10.8)	2 (2.2)	1 (1.07)	1 (1.070)	93 (43.3)	258 (30.9)
26–30	40 (48.2)	31 (37.4)	9 (10.8)	2 (2.4)	1 (1.2)	0	83 (38.6)	323 (38.7)
31–35	4 (40)	4 (40)	2 (20)	0	0	0	10 (4.65)	50 (6)
36–40	4 (50)	4 (50)	0	0	0	0	8 (3.7)	47 (5.6)
41–45	3 (50)	2 (33.3)	0	1 (16.7)	0	0	6 (2.8)	34 (4.1)
46–50	1 (25)	3 (75)	0	0	0	0	4 (1.9)	26 (3.1)
51–55	0	1 (50)	0	0	0	1 (50)	2 (0.9)	29 (3.5)
55–60	0	0	0	0	0	1 (100)	1 (0.5)	24 (2.9)
Total	101 (46.9)	79 (36.7)	22 (10.2)	6 (2.8)	3 (1.4)	4 (1.9)	215 (20.5)	835 (79.5)

Percentages are given in parenthesis. Total no. of patients studied = 1050.

Table 2
Signs/symptoms of patients and their correlation with culture positivity ($n = 1050$).

Signs/symptoms	Number		*P* value
	Culture positive (n) = 215	Culture negative (n) = 835	
Pruritis with or without discharge	172 (80%)	442 (52.9%)	<0.001
Vaginal erythema	160 (74.4%)	525 (62.9%)	<0.001

Significant *P* value is <0.05.

than those with *C. albicans* infection. Our findings are consistent with those of previous work [9]. This is possibly due to the fact that *C. glabrata* is associated with estrogen hormone depletion and elevated pH levels in the vagina of postmenopausal women; it also suggests that *C. albicans* is more susceptible to the variation in estrogen hormones than *C. glabrata* [7,12].

Our microscopic study for yeast cells and pseudohyphae was found to be positive in 167 of the 215 women with positive culture (77.67%). Moreover, earlier workers [16] support these findings. The difference in the sensitivity of direct microscopy may be due to the difference in the concentration of yeast in different vaginal secretions [10,17]. Direct microscopy is acceptable only if the infection is severe. Therefore, if microscopy fails to diagnose properly, vaginal swab cultures are important for testing for VVC. The patient should be recommended for the vaginal swab culture before starting antifungal therapy.

In the present study, *C. albicans* was the most frequently isolated (46.9%) of all six *Candida* species, which has also been reported by other co-workers [14,18], whereas some other authors have reported *C. glabrata* to be a predominant species [19,20]. The percentage occurrence of VVC due to non-albicans species (53.04%) was higher compared with albicans species (46.9%) in the present study, which is consistent with the earlier studies [6,9]. The maximum occurrence of non-albican *Candida* reported is that of *C. glabrata*, which is comparable to the results of our findings [6]. These non-albicans *Candida* species are comparatively nonpathogenic, but eventually they are elected, and begin emerging frequently due to the extensive misuse of over the counter antifungals, the use of single-dose oral and topical azoles treatments, and the long-standing protection treatments of oral azoles [21,22].

Table 3
Potential risk factors for vulvovaginal candidiasis.

Risk factors	No. of patients studied[a]	No. (%) of patients positive[b]	*P* value[**]
Pregnancy			
Pregnant	250	190 (76)	<0.001
Nonpregnant	800	25 (31)	
Parity			
P0	350	70 (20)	<0.001
P1	500	50 (10)	
>P2	200	95 (47.5)	
Oral contraceptives			
Users	200	115 (57.5)	<0.001
Non-users	850	100 (11.8)	
Antibiotics			
Users	250	112 (44.8)	<0.001
Non-users	800	103 (12.9)	
Age group			
15–20	52	8 (15.4)	
21–25	341	83 (24.3)	
26–30	416	93 (23.4)	
31–35	60	10 (16.7)	
36–40	55	8 (14.5)	
41–45	40	6 (15)	
46–50	30	4 (13.3)	
51–55	31	2 (6.5)	
56–60	25	1 (4)	
Genital hygiene			
Satisfactory	800	125 (15.6)	<0.001
Unsatisfactory	250	90 (36)	
Educational standard			
Illiterate	750	155 (20.7)	0.8
Literate	300	60 (20)	

[a] Total no. of patients studied = 1050.
[b] Patients showing positivity = 215.
[**] Significant *P* value is <0.05.

The maximum frequency of *Candida*-positive cultures in the 21–25 age group, closely followed by the 26–30 age group, supports the reports of some other workers [12,18,23]. The probable reason is active sexual relations in this age group, which may cause frequent genital candidiasis. Other co-workers have also reported the lowest frequency of *Candida*-positive cultures in women aged above 41 years [12].

C. albicans was predominantly isolated from women of childbearing age (21–45 years), which is also supported by the findings of others [18,23]. The possible reason is glycogen, which is deposited due to the secretion of the estrogen hormone and provides a favorable environment for the growth of *C. albicans*. There is a report on the maximum frequency of occurrence of *C. glabrata* in an advanced age group (45–55 years) [12], whereas *C. parapsilosis* is found to be the second most prevalent non-albicans *Candida* species after *C. glabrata* followed by *C. tropicalis*, *C. krusei*, and *C. kiefer* [24]. The increase in the frequency of non-albicans species is due to the drastic changes in the hormonal balance during the menopause phase and the treatment of estrogen hormones, aging, which will affect the immunity, and a long stay in the hospital wards [25]. Thus, while testing the vaginal samples, non-albicans species should also be taken into consideration for the proper diagnosis of abnormal conditions.

Among the various symptoms, the presence of pruritus, in culture-positive and culture-negative women, was statistically significant ($P < 0.001$). The findings of the present study are in agreement with those of other authors [9,26]. Some authors reported 72% pruritus in culture-positive women and 47% in culture-negative women [27]. The women suffering from VVC have the complaint of a burning sensation of the vulval epithelium, which is due to the metabolites of yeast and sometimes due to vulvar skin infection [9]. Antifungal therapies to the vulva not only facilitate infections, but also worsen contact dermatitis, which is another of the complaints. Vulvar areas may present mixed infection like vulvar dermatitis. Vaginal erythema was significantly higher in culture-positive women than in culture-negative women ($P < 0.001$). Some other authors also reported significantly more complaints of vaginal erythema compared with culture-negative women [9,26].

The study of various potential risk factors showed that *Candida* positivity was significantly associated with pregnancy and an increase in parity was also reported by other authors [6,9]. *C. albicans* was more frequently isolated from the pregnant women compared with non-pregnant women. This may be due to the opportunistic nature of *C. albicans*. It is suggested that during pregnancy there is high glycogen content in the vaginal tissues due to the high level of hormones. Glycogen provides an excellent

source of carbon for *Candida* [6]. Oral contraceptives have also previously been reported to be a predisposing factor for VVC [9,17]. This is because of the high levels of estrogen occurring as the oral contraceptive increases the colonization of *Candida* in the vagina [17].

Candida infection was found to be common among women using antibiotics ($P < 0.001$). Many authors have also reported high incidences of VVC in women using antibiotics [9,16]. The utilization of antibiotics may also intensify the symptoms by inactivating the defensive vaginal flora [28].

Women with unsatisfactory genital hygiene showed significantly higher incidence of VVC than those with satisfactory genital hygiene ($P < 0.001$), as has also been shown by other workers [9,16].

5. Conclusion

The culture positivity of *Candida* species in 20.47% of women suggests that vulvovaginal candidiasis cannot be diagnosed only by using clinical criteria; rather, vulvovaginal symptoms and *Candida* cultures are also required. The incidence of VVC caused by albicans and non-albicans species are common in women of reproductive age. The important relationship between VVC and certain epidemiological factors emphasizes the need to educate women regarding genital hygiene, precise diagnosis, and punctual treatment.

Acknowledgements

We are thankful to the central instrumentation facility of the IBU, AMU. This work was supported by the CSIR sanction no. 37(1209)04 EMR II to AUK. The author is grateful to Prof. S. Akhtar Husain, JMI New Delhi, Prof. Zakia Arshad, and Dr. Naheed Khan for their valuable suggestions.

References

[1] Ventolini G, Baggish MS. Recurrent vulvovaginal candidiasis. Clin Microbiol Newsl 2006;28:11.
[2] Spacek J, Buchta V, Jílek P, Förstl M. Clinical aspects and luteal phase assessment in patients with recurrent vulvovaginal candidiasis. Eur J Obstet Gynecol Reprod Biol 2007;131:198–202.
[3] Moreira D, Paula CR. Vulvovaginal candidiasis. Int J Gynecol Obstet 2006;92:266–7.
[4] Fidel Jr PL. History and new insights into host defense against vaginal candidiasis. Trends Microbiol 2004;12:220–7.
[5] Goplerud C, Ohm M, Galask R. Aerobic and anaerobic flora of the cervix during pregnancy and the puerperium. Am J Obstet Gynecol 1976;126:858.
[6] Sobel JD, Faro S, Force RW, et al. Vulvovaginal candidiasis; epidemiologic, diagnostic and therapeutic consideration. Am J Obstet Gynecol 1998;178: 203–11.
[7] Sobel JD. Vulvovaginitis when candida becomes a problem. Sex Transm Dis 1998;16:763–8.
[8] Fidel PL, Barousse M, Espinosa T, et al. An intravaginal live Candida challenge in humans leads to new hypotheses for the immunopathogenesis of vulvovaginal candidiasis. Infect Immun 2004;72:2939–46.
[9] Tarry W, Fisher M, Shen S, Mawhinney M. *Candida albicans*: the estrogen target for vaginal colonization. J Surg Res 2005;129:278–82.
[10] Jindal N, Gill P, Aggarwal A. Significance of candida culture in women with vulvovaginal symptoms. J Obstet Gynecol India 2006;56:139–41.
[11] Okungbowa FI, Dede AP, Isikhuemhen OS, Okungbowa MO. Age and marital distributions of genitourinary candidiasis among symptomatic women in Nigeria. Med J Islam World Acad Sci 2006;16:67–9.
[12] Okungbowa FI, Isikhuemhen OS, Dede AP. The distribution frequency of Candida species in the genitourinary tract among symptomatic individuals in Nigerian cities. Rev Iberoam Micol 2003;20:60–3.
[13] Boon C, Deng Y, Wang LH, He Y, Xu JL, Fan Y, Pan SQ, Zhang LH. A novel DSF-like signal from Burkholderia cenocepacia interferes with *Candida albicans* morphological transition. ISME J 2008;2:27–36.
[14] Simhan HN, Caritis SN, Krohn MA, Hillier SL. Elevated vaginal pH and neutrophils are associated strongly with early spontaneous preterm birth. Am J Obstet Gynecol 2003;189:1150–4.
[15] Abd-El-Maeboud KH, Ghazy AA, Nadeem AA, Al-Sharaky A, Khalil AE. Effect of vaginal pH on the efficacy of vaginal misoprostol for induction of midtrimester abortion. J Obstet Gynaecol Res 2008;34:78–84.
[16] Jindal N, Gill P, Aggarwal A. An epidemiological study of vulvovaginal candidiasis in women of childbearing age. Indian J Med Microbiol 2007;25:175–6.
[17] Consolaro MEL, Albertoni TA, Yoshida CS, Mazucheli J, Peralta RM, Svidzinski TIE. Correlation of *Candida* species and symptoms among patients with vulvovaginal candidiasis in Maringá, Paraná Brazil. Rev Iberoam Micol 2004;21:202–5.
[18] Vermitsky JP, Self MJ, Chadwick SG, Trama JP, Adelson ME, Mordechai E, Gygax SE. A survey of vaginal-flora *Candida* species of different age groups using species-specific PCR detection. J Clin Microbiol 2008;46(4):1501–3.
[19] Goswami D, Goswami R, Banerjee U, Dadhwal V, Miglani S, Lattif AA, Kochupillai N. Pattern of Candida species isolated from patients with diabetes mellitus and vulvovaginal candidiasis and their response to single dose oral fluconazole therapy. J Infect 2006;52:111–7.
[20] Ilkit M, Hilmioglu S, Tasbakan M, Aydemir S. Evaluation of Albicans ID2 and Biggy agar for the isolation and direct identification of vaginal yeast isolates. J Med Microbiol 2007;56:762–5.
[21] Kent HL. Epidemiology of vaginitis. Am J Obstet Gynecol 1991;165:1168–76.
[22] Dennerstein G. Review. The treatment of candida vaginitis and vulvitis. JAMA India 2001;4:50–2.
[23] Akinbiyi AA, Watson R, Feyi-Waboso P. Prevalence of *Candida albicans* and bacterial vaginosis in asymptomatic pregnant women in South Yorkshire. United Kingdom. Outcome of a prospective study. Arch Gynecol Obstet 2008;278(5):463–6.
[24] Tabrizi SN, Pirotta MV, Rudland E, Garland SM. Detection of Candida species by PCR in self-collected vaginal swabs of women after taking antibiotics. Mycoses 2006;49:523–4.
[25] Pfaller MA, Diekema DJ, Messer SA, et al. Activities of fluconazole and voriconazole against 1586 recent clinical isolates of Candida species determined by broth microdilution, disk diffusion, and Etest methods: report from the ARTEMIS Global Antifungal Susceptibility Program, 2001. J Clin Microbiol 2003;41:1440–6.
[26] Bedout C, Gibbs DL. The Global Antifungal Surveillance Group. Comparison of the susceptibilities of *Candida* spp. to fluconazole and voriconazole in a 4-year global evaluation using disk diffusion. J Clin Microbiol 2003;41:5623–32.
[27] Luo G, Mitchell TG. Rapid identification of pathogenic fungi directly from cultures by using multiplex PCR. J Clin Microbiol 2002;40:2860–5.
[28] Sobel JD. Vulvovaginal candidiasis. In: Sexually transmitted diseases. New York: McGraw-Hill; 1999. p. 629–37.