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Clinical patterns and risk factors of vulvo-vaginal candidiasis among women of reproductive age attending a tertiary hospital in central India

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Vulvo-vaginal Candidiasis (VVC) is the most common fungal infection in women of reproductive age. Data related to distribution and risk factors are very limited in India. This study was designed to observe the prevalence of VVC among women of reproductive age group, find the species of *Candida* causing such infection and find the risk factors associated with VVC. All female patients in the childbearing age group, fulfilling the clinical criteria of vaginitis, reported between January 2016 and June 2017 were included in this study. Standard procedures were followed to collect vaginal swabs. Culture and microscopic examinations were done to isolate *Candida albicans* and non-*albicans Candida* (NAC) from the specimens. Descriptive and analytic statistics was used to illustrate the basic and disease characteristics of the study participants. The odds-ratio (OR) associated with each potential risk factor at 95% confidence interval (CI) were calculated. All results were considered significant at *P*<0.05. Out of total 168 subjects, 32.7% showed pure growth of *Candida* species and NAC species were found to be predominant (65.4%) followed by *C. albicans* (34.6%). Maximum *Candida* positivity was found in age group 21-30 years (60%) compared to other age groups (*P*<0.05). *Candida* positivity was found to be higher among pregnant (45.0%) than non-pregnant (28.9%) women (*P*<0.05) suggests that pregnant women are twice at odds for developing VVC as compared to non-pregnant women. The higher positivity in patients of vaginal discharge with pruritus was found to be statistically significant (*P*<0.05). One in three patients was found to be positive for VVC and NAC was more prevalent as compared to *candida albicans*. This study concluded that pregnant women are at risk for VVC.

Keywords: Vulvo-vaginal candidiasis, *C. albicans*, non-*albicans Candida* (NAC), Pregnancy.

INTRODUCTION

Vulvo-vaginal Candidiasis (VVC) is defined as a condition with signs and symptoms of inflammation in the presence of *Candida* species in spite of other etiology (1). Candida is believed to be one of the most common causes of vaginal infections accounting for an estimated 17% to 39% cases (2). Approximately 75% of women experience at least one episode of VVC during their life (3). However, an estimated 5% of women with vulvo-vaginal candidiasis experience recurrent vulvo-vaginitis (RVV), which is defined as four or more distinct episodes of vaginitis in a single year (4, 5).

Women having VVC present with spectrum of manifestations ranging from asymptomatic colonization to severe acute symptomatic infection (6). Certain patients may develop primarily vulvar symptoms instead of vaginal manifestations of VVC. Vulval pruritus and burning are the main symptoms and these are frequently accompanied by soreness, irritation, dyspareunia and dysuria. Vulval and vaginal erythema, edema, fissures and thick vaginal discharge are other common symptoms (7).

Prevalence of VVC increases in certain groups, such as pregnant or diabetic women, those using oral contraceptives and after antibiotic treatment (8). The incidence of candidiasis is almost doubled in pregnant women particularly in the third trimester compared to the non-pregnant women (9). VVC in the expectant women can cause abortion, chorio-amnionitis and subsequent preterm delivery. Premature neonates are severely endangered by generalized fungal infection because of their immature immune system (10).

VVC is the most common fungal infection in the world and any information on its distribution and etiology is useful for epidemiological purpose. Data related to this context is very limited in our region.

MATERIALS AND METHODS

Sampling criteria. This observational cross-sectional study was carried out in the Department of Microbiology, at a tertiary care hospital of central India between January 2016 and June 2017. All female patients in the reproductive age group (15-49 years) fulfilling the clinical criteria of vaginitis i.e. those with vaginal discharge, irritation, itching with or without pain and willing to give consent for the study were invited to participate. Vaginal swabs showing evidence of bacterial and protozoal infection and patients not willing to give consent were excluded. Consecutive sampling was followed to reach the desired sample size of 168.

Specimen and tests. Three high vaginal swabs were collected from each patient using sterile cotton-tipped swabs. Collected specimen was transported immediately (within 30 minutes) to the laboratory in sterile tubes without using transport media. One swab was used for wet mount and second one for Gram staining (11). Third swab was used for culture on Sabouraud's Dextrose agar (SDA). Inoculated SDA culture tubes were incubated at 25°C for three to four days. Identification of culture growth was by colony characteristics (smooth, white to cream colored colonies were identified as those of candida); Gram's staining (round to oval Gram positive budding cells with or without pseudo-hyphae were considered as positive for candida). Germ tube test was carried out as a confirmatory test for *Candida* spp**.** For

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species identification various tests were used including Chlamydospore formation, sugar fermentation test, growth on CHROM agar (BD Diagnostic Systems Europe, Heidelberg, Germany) as described in previous studies (12, 13).

Statistical analysis. Data was analysed using SPSS version 20. Descriptive statistics in form of frequencies and percentages were used to illustrate the basic and disease characteristics of the study population. For quantitative analysis, univariate analysis using chi-square test and Fisher's exact test was used. The odds-ratio (OR) associated with each potential risk factor and the 95% confidence interval (CI) were calculated. All results were considered significant at *P* values less than 0.05.

RESULTS AND DISCUSSION

A total of 168 subjects were included in the study. Of these, 76.2% subjects were non-pregnant and 23.8% were pregnant (Table 1). Among the 168 subjects, vaginal swab of 55 (32.7%) subjects showed pure growth of *Candida* species (Figure 1). Table 2 represents distribution of *Candida* species. The non-*albicans Candida* (NAC) species were predominant with 65.4%. The most common species isolated on the basis of colony characteristics, morphology on corn meal agar, germ tube and chlamydospore formation, fermentation and assimilation reactions was *C. albicans* (34.6%) followed by *C. tropicalis* (23.6%) and *C. glabrata* (21.8%).

Table 1. Basic characteristics of the study subjects

Variable		Frequency	Percentage
Age	Upto 20	8	4.76
	21-30	82	48.81
	31-40	61	36.31
	More than 40	17	10.12
Pregnancy status	Pregnant	40	23.80
	Non-Pregnant	128	76.20
Risk factors	None	59	35.12
	Diabetes	3	1.78
	Gravida>1	86	51.20
	BOH	13	7.73
	OCP use	5	2.97
	BOH+DM	2	1.2

BOH = Bad obstetric history; DM = Diabetes mellitus; OCP = Oral contraceptive pill.

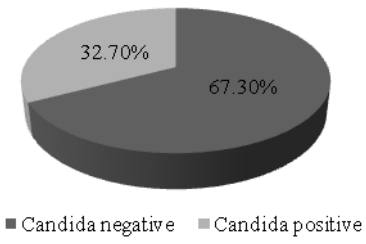

Table 2. Distribution of *Candida* species isolated from study participants (n=55)

***Candida* species**	**n (%)**
C.albicans	19 (34.6)
Non *albicans*	36 (65.4)
C. dublinensis	04 (7.3)
C. glabrata	12 (21.8)
C. guilliermondi	01 (1.8)
C. krusei	01 (1.8)
C. lusitaniae	03 (5.5)
C. parapsilosis	02 (3.6)
C. tropicalis	13 (23.6)

Table 3 shows the *Candida* positivity across different age groups. Maximum *Candida* positivity was seen in age group 21-30 years (60%) followed by age group 31-40 years (32.72%). The higher positivity in age group 21-30 years was statistically significant in comparison to other age groups ($P < 0.05$). Table 4 presents information on the effect of risk factors on *Candida* positivity. Isolation of *Candida* was higher in women with one or more risk factors (94.5%) as compared to those without any risk factors. *Candida* positivity was found to be higher among pregnant (45.0%) as compared to non-pregnant (28.9%) subjects included in the study. This difference in positivity among pregnant and non-pregnant women was not statistically significant ($P > 0.05$). The odds ratio suggests that pregnant women are twice at odds for developing VVC as compared to non-pregnant women. *Candida* positivity in patients having history of diabetes mellitus together with bad obstetrical history was 100%. This was followed by that in patients presenting with BOH alone (61.5%), pregnancy (45%), diabetes mellitus alone (33.3%), multigravida (25.6%) and those taking oral contraceptives (20%). However, none of these differences were statistically significant.

Table 5 shows signs and symptoms in study participants. Vaginal swab of 54 (96.4%) out of 56 subjects presenting with thick curdy white discharge is the characteristic of vaginal candidiasis that were positive for *Candida*, in contrast to only 1 (0.9%) of 112 subjects presenting with thin white non-curdy discharge and this difference was found to be significant statistically ($P < 0.05$). Isolation of *Candida* was the highest in women presenting vaginal discharge with pruritus (56.9%), followed by those with dyspareunia or pruritus, dysuria and discharge (33.3% each). Dysuria with vaginal discharge

Table 3. Distribution of *Candida* by age

Age group in years	**No. of subjects, n (%)**	**Positive for Candida, n (%)**	**Percentage positivity**
Up to 20	08 (4.76)	02 (3.64)	1.20
21-30	82 (48.81)	33 (60.0)	19.64
31-40	61 (36.31)	18 (32.72)	10.71
More than 40	17 (10.12)	02 (3.64)	1.20
Total	168	55	32.75

accounted for 25% positivity and vaginal discharge alone for 20.2% *Candida* positivity. The higher positivity in patients of vaginal discharge with pruritus was found to be statistically significant ($P < 0.05$). Thick curdy white discharge was found to be associated with confirmed vaginal candidiasis in 96.3% of such subjects. Additional presence of either excoriation or vulval swelling further strengthened the diagnosis, as 100% of such subjects grew *Candida* spp.

Table 4. Effect of risk factors on *Candida* positivity

Risk factors		Candida positive n (%)	Candida negative	OR and CI 95%	*P* value
Risk factor Present	149	52 (34.9)	97 (65.1)	2.8591	0.1072
Risk factor Absent	19	3 (15.8)	16 (84.2)	[0.7963 to 10.2660]	
Type of risk factor					
Pregnancy					
Yes	40	18 (45.0)	22 (55)	2.0123	
No	128	37 (28.0)	91 (72)	[0.9690 to 4.1790]	0.0607
Gravida>1				0.8818	
Yes	86	22 (25.6)	64 (74.4)	[0.4453 to 1.7462]	0.7182
No	82	23 (28.1)	59 (71.9)		
BOH					
Yes	13	8 (61.5)	5 (38.5)	**	
No	155	47 (30.4)	108 (69.6)		
DM					
Yes	3	1 (33.3)	2 (66.6)	**	
No	165	54 (32.7)	111 (67.2)		
OCP					
Yes	5	1 (20.0)	4 (80)	**	
No	163	54 (33.2)	109 (66.8)		
BOH+DM					
Yes	2	2 (100)	0 (0)	**	
No	166	53 (32.0)	113 (68.0)		

BOH = Bad obstetric history; DM = Diabetes mellitus; OCP = Oral contraceptive pill
** Fisher exact test was used

Table 5. Signs and symptoms in study participants

Symptoms	No. of subjects, n=168 (%)	Candida positive, n=55 (%)
Vaginal discharge alone	94 (55.95)	19 (34.55)
Vaginal discharge + Pruritus	51 (30.36)	29 (52.72)
Vaginal discharge + Dysuria	08 (4.76)	02 (3.64)
Vaginal discharge + Dyspareunia	09 (5.36)	03 (5.45)
Vaginal discharge + Pruritus + Dysuria	06 (3.57)	02 (3.64)
Signs		
Thick curdy white discharge alone	54 (32.14)	52 (94.5)
Thin non-curdy white discharge alone	109 (64.88)	1 (1.81)
Thick curdy white discharge + Excoriation	01 (0.59)	1 (1.81)
Thin non-curdy white discharge + Excoriation	02 (1.19)	0 (0)
Thick curdy white discharge + Vulval swelling	01 (0.59)	1 (1.81)
Thin non-curdy white discharge + Vulval swelling + Excoriation	01 (0.59)	0 (0)

In the present study, VVC was found in about one in three of the symptomatic women. This is in concordance with two reports from India that is 31.1%, and 37% on a similar study group (14, 15). Onifade *et al*. (16) from Nigeria (81.5%) reported a very high prevalence. These differences may be attributed to several factors including the type of patient studied, sample size, source of clinical specimens and presence of risk factors.

Recently, NAC species have been recovered with increasing frequency. In our study, the rate of isolation of NAC was higher than that of *C. albicans*. However, *C. albicans* was the most dominant species isolated. Higher isolation of NAC over *C. albicans* has also been reported by Kikani *et al*. (55.6% vs. 44.4%) (17), Babin *et al*. (64.5% vs. 35.5%) (18) and Namrata *et al*. (53% vs. 47%) (19). Earlier reports from Middle East region revealed higher rate of isolation of *C. albicans*. Cultural, ethnic and epidemiological differences may influence the isolation rate of different yeast from vulvo-vaginitis samples (20-23). However, it is critically important to isolate and identify *Candida* species for diagnosis, treatment and epidemiological purposes.

The age group, 21-30 years is the most sexually active age group. There is high risk of pregnancy, indiscriminate use of drugs, use of contraceptive pills and other protective family planning devices to prevent pregnancy. AIDS and other sexually transmitted diseases are frequent in this age group. All these factors promote VVC and can be a reason for higher prevalence of VVC in this particular age group. Another physiological reason is that during this period, the ovary produces high amount of estrogen, which favors the growth of *Candida* by maintaining the acidic pH and enhancing the yeast adherence to vaginal epithelial cells. In the present study, incidence of VVC in age group 21-30 years was 40.2 %. The higher incidence of VVC in the age group of 20-30 years was also reported in previous reports (24-28). A similar case study in Libya, demonstrated the highest positivity in age group 15-25 years (44.4%) which

was found to be associated with early age of marriage (29).

Vaginal discharge alone was reported to be the predominant sign/symptom observed in cases of VVC by other workers (30-34).In this study we found 20.2% cases with thick cheesy vaginal discharge which is similar to previous reports (30,31). Maximum *Candida* positivity in the present study was seen in patients with thick curdy white discharge (96.4%) as compared to thin non-curdy white discharge (0.9%) and this difference was statistically significant ($P < 0.05$). Fule *et al*. (15) found this to be the major symptom in 52.4% of their subjects of VVC and Ugwa *et. al*. (32) found this to be the most common symptom in 47.4% of their cases. *Candida* positivity in patients presenting vaginal discharge plus one or more of the other symptoms was 48.7% in this study which is similar to another report (47.5%) by Fule *et al*. (15).

Vaginal discharge with pruritus (56.9%) was a major symptom in *Candida* positive cases in the present study ($P < 0.05$). Similar result was reported by other researchers and demonstrated in 63.2% cases (31). Dysuria with pruritus was seen in 33.3% cases and dysuria alone in 25% *Candida* positive cases was observed in the present study. Vaginal discharge with dysuria was also observed in 18% (10) and 12% (33) cases in other studies which is less than our findings. One third of our cases presenting with dyspareunia were positive for *Candida* which is higher than another previous report (33).

On analyzing the predisposing risk factors, in the present study we observed that VVC was confirmed in 45% of pregnant women whereas in non-pregnant women it was confirmed in 28.9% cases and this difference was not statistically significant. Several previous studies reported a similar finding of higher prevalence in pregnant as compared to non-pregnant (24, 29, 30, 35). Another study also reported pregnancy to be the most typical predisposing risk factor (29.75%) for VVC (18) and also, been cited by other researchers (33, 36). Increased level of vaginal glycogen, high level of reproductive hormones and immune-suppression during pregnancy has been attributed as important risk factors for VVC. Emotional stress during pregnancy has also been incriminated as a major risk factor for VVC.

Several studies (19, 37, 38) found oral contraceptive pill OCP to be a risk factor for VVC. Our observation of association of oral contraceptive as predisposing factor was seen in 20% cases. Estrogen facilitates the adherence of yeast to the vaginal epithelium; hence high estrogen in oral contraceptives may be one of the contributing factors.

In the present study, 33.3% of women with diabetes mellitus alone had confirmed VVC and 100% of those who had diabetes mellitus along with bad obstetrics history confirmed VVC. These results show resonance with previous studies (39-41). Earlier studies on diabetic women in developed countries demonstrated wide variations in prevalence rates starting from 7% to >50% (42-44). In diabetic patients, especially among type I diabetes, hyperglycemia limits neutrophil function. The neutrophils are unable to phagocytose and kill *Candida* and thus there is a higher incidence of candidiasis in diabetes patients. Diabetes is a proven predisposing factor for vulvo-vaginal infection especially for vaginal candidiasis, along with pregnancy, use of broad-spectrum antibiotics, high-estrogen-dose, oral contraceptives, obesity and drug addiction (45).

In the present study, 25.6% of multigravidas were positive for VVC. Other studies have reported a significantly higher prevalence in multigravidas as compared to primigravida women (33, 46).

The limitation of our study was its small sample size which was collected from just one tertiary care hospital in central India. Due to this constraint, the results of this study cannot be generalized. However, this study provides important information about the pattern and risk factors of VVC infection in women of reproductive age in central India. It is recommended to take up further study of *C. albicans* and NAC as important pathogens, especially in high risk groups.

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