

Original Research Article

Risk Factors Associated With Vulvo vaginal Candidiasis among Women in a Rural Community in Western Uttar Pradesh, India

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Abstract: The objective is to determine risk factors of vulvo vaginal candidiasis (VVC) in women of a rural community. A total of 322 women comprised of 44 HIV-infected, 177 HIV negative and 101 healthy women were included in this study. Diagnosis of VVC was done by estimation of vaginal pH, microscopic examination of vaginal swabs and culture test for *Candida* species. Statistically significant association was identified between the age group ≤ 40 years ($p < 0.0001$; Odd ratio (OR) = 56.4), previous antibiotics users ($p < 0.0001$; OR = 4.37),azole users ($p = 0.0054$; OR = 2.29), AIDS patients ($p = 0.0003$; OR = 3.74), HAART (patients those do not received) ($p = 0.0002$; OR = 0.06), CD4 count < 200 cell/mm³ ($p = 0.0023$; OR = 3.08), working women ($p = 0.0001$; OR = 62.13) and patients belongs to poor economic status ($p = 0.0003$) with VVC. Patients without medical awareness were found frequently associated with VVC, though without a significant relationship. Patients with pruritus vagina and burning during micturition were more prone to *Candida* infection. HIV-infected women having vaginal secretions like white color, malodorous with curdy character and thick consistency showed higher *Candida* carriage than HIV negative patients. Our results are important for the development of strategies to eliminate these indicators of risk and significantly reduce VVC.

Keywords: Vulvo vaginal candidiasis, rural community, symptoms, *Candida* culture positive, HIV-infected, *Candida albicans*

INTRODUCTION

Vulvo vaginal candidiasis (VVC) is a common fungal infection that affects healthy women of all ages. At least 75% of women will develop one or more infections once during their lifetime, with 5 to 8% of those individuals developing recurrent infections [1, 2]. Possible risk factors causing an increase in *Candida* infections include prior antibiotic therapy, pregnancy, diabetes mellitus, oral contraceptives containing estrogen and progestin, and immunosuppressed patients (transplanted patients, cancer patients treated with chemotherapy, and HIV patients[3].Although vaginal colonization with *Candida* appears to be greater in HIV-seropositive women than in HIV-seronegative women, symptomatic vaginal candidiasis is common in women, regardless of HIV serostatus [4,5].The principal symptoms of *Candida* vulvovaginitis are vulvar and/or vaginal pruritus and a thick vaginal discharge although these complaints are very nonspecific[6].

In practice, many Indian women who self-diagnosed for immediate and personal treatment of presumed vulvo vaginal candidiasis use intravaginal preparations of butaconazole, clotrimazole, miconazole and boric acid in gelatine capsules, since these medicines are available over-the-counter (OTC). The lack of specificity of symptoms and signs precludes a diagnosis that is based on history and physical examination without the corroborative evidence of laboratory tests [6]. Although VVC is both treatable and mild, when left untreated, is a possible risk for acquisition of HIV/AIDS as well as other complications. It is now well established that the presence of infective vaginal discharge greatly facilitates transmission and acquisition of HIV between sexual partners [7]. Therefore, there is a need for prevention, early diagnosis and prompt treatment of this common condition especially among the risk groups, in order to avert the complications and reduce the transmission of HIV. We planned this study, in which all steps starting from patient's selection, filling proforma, examination of signs and symptoms, vaginal

secretion examination, HIV status and vaginal swab culture were carried out to gather the real state of risk factors of vulvovaginal candidiasis in women belongs to a rural community. All the women in our study belonged to rural area of Meerut and Muzaffarnagar, India therefore medical awareness and socio economic status are also important factors determining the prevalence of vulvovaginal candidiasis.

MATERIALS AND METHODS

Study population

This prospective study was conducted from January 2006 to December 2010; in which vaginal fluid samples were collected from 221 women with complaints of vaginal discharge, vaginal itching and irritation. Amongst 221 women, 44 were HIV-infected (considered as study Group 1) and 177 were HIV negative (considered as study Group 2). These patients were recruited from outpatients of Gynaecology Department of Lala Lajpat Rai Memorial Medical College and Hospital, Meerut, India. Control group consisted of 101 healthy women, without symptoms of vulvovaginitis and without recent treatment with antibiotics/and or antifungal agents. Risk factors for VVC included in this study were age group, antibiotics received,azole received, CD4 count < 200 cells/mm³, AIDS diagnosed, patients those do not received HAART, medical awareness, occupation, socio economic status of patients. All women were clinically examined by Gynaecologist for the clinical signs and symptoms of VVC. The data obtained from clinical history of each patient was following: previous antibiotic and antifungal treatment, CD4 counts and treatment with highly active antiretroviral therapy (HAART).

Specimen Collection and processing

Two sterile, cotton tipped swabs were used to collect specimens from lateral wall of vagina of each women. One of the swabs was used to determine the presence of yeast by direct wet mount microscopy using a drop of 10% potassium hydroxide (KOH) solution. The material on the other swab was transferred onto the culture plate containing Sabouraud's dextrose agar (Hi-Media, Mumbai) supplemented with 0.06 µg/mL gentamycin, with and without cycloheximide (0.5%). Plate was incubated for 48 h at 37°C. The pasty, opaque and pale coloured colonies that developed on incubation were subjected to germ tube test and corn meal agar test [8]. Biochemical tests were performed, using HI *Candida* KB006 Kit (Hi-Media, Mumbai, India) containing sterile media for urease production and

different carbohydrate utilization test. Each well on plate containing reference carbohydrate was inoculated with 50 µl of the inoculum (2.5×10^3 CFU/ml), and incubated at 25°C for 24-48 h. Change in color indicates assimilation of the respective carbohydrates [9]. HIV sero status of the individuals was tested according to NACO guidelines using three rapid tests based on different principles (Tridot Biomed Industries, COMB Elisa Span Diagnostic Ltd., HIV Capillus, Trinity Biotech Plc.).

STATISTICAL ANALYSIS

The statistical analysis was performed using Graph Pad Prism 6. Univariate analyses were performed on all variables of this study using the Fisher's and Chi-squared tests (2-sided tests). The results of this analysis were expressed as an odds ratio (OR) with a 95% confidence interval (CI). A *p*-value of < 0.05 was taken as indicative of statistical significance and *p*-value of < 0.01 was considered highly significant.

RESULTS

Patient's demography

The sample included 44 (Group 1) and 177 (Group 2) women with symptoms of VVC. Age varied from 19 to 49 years (with mean age of 33.7 years). Regarding the risk factors, the sample included 37.1% (82/221) patients who had previously used antibiotics, 41.2% (91/221) previously used azoles, 18.1% (40/221) were AIDS diagnosed, 17.6% (39/221) had CD4 count < 200 cells/mm³, 50.0% (22/44) were without HAART (patients those /do not received), 55.7% (123/221) were medically unaware, 31.7% (70/221) were working and 59.3% (131/221) belongs to poor economic status.

Candida culture positive and species distribution

Out of 221 patients, 71 (32.1%) were *Candida* culture positive. Amongst 44 Group 1 patients, 52.3% (23) were *Candida* culture positive while 27.1% (48) of 177 Group 2 women were *Candida* culture positive. In the control group only 10.9% women showed *Candida* colonization (Table 1). *C. albicans* was the most common isolate from all the study groups. Non-*albicans Candida* (NAC) isolates were 28.0% (7/25) and 31.4% (16/51), in Group 1 and Group 2 patients respectively. The frequency of isolation of *Candida* in Group 1 was: *C. albicans* 72.0%, *C. glabrata* 20% and *C. krusei* 8% while in Group 2 was: *C. albicans* 68.6%, *C. tropicalis* 9.8%, *C. glabrata* 17.6%, *C. krusei* 1.9%, and *C. guilliermondii* 1.9%. Control group presented only two *Candida* species constituted 10 isolates of *C. albicans* and two isolates of *C. tropicalis* (Table 2).

Table 1: Candida isolation rate from all groups

Study Groups (n)	Candida carriage n (%)	Candida isolates (n)
Group 1* (44)	23 (52.3)	25
Group 2† (177)	48 (27.1)	51
Test Group‡ (221)	71 (32.1)	76
Control group (101)	11 (10.9)	12

Note. Data are shown in no. (%) of *Candida* carriage; * HIV-infected patients with symptoms of VVC; † HIV-negative patients with symptoms of VVC; ‡ All HIV-infected and non-infected patients.

Table 2: Distribution of Candida species in different groups

Species	Group 1* (n=25)	Group 2† (n=51)	Control group (n=12)
<i>C. albicans</i>	16 (64.0)	32 (62.7)	9 (75.0)
<i>C. tropicalis</i>	-(0)	3 (5.9)	1(8.3)
<i>C. glabrata</i>	5 (20.0)	9 (17.6)	-(0)
<i>C. albicans</i> + <i>C. tropicalis</i>	-(0.0)	2 (3.9)	1(8.3)
<i>C. albicans</i> + <i>C. krusei</i>	2 (8.0)	1 (1.9)	-(0)
<i>C. guilliermondii</i>	-(0)	1 (1.9)	-(0)
NAC isolates	7 (28.0)	16 (31.4)	2 (16.7)

Data are shown in no. (%) of *Candida* isolates; * HIV-infected patients; † HIV-negative patients; n, number of isolates recovered; NAC, non-*albicans* *Candida*.

Evaluation of risk factors

Table 3 summarizes the proportional prevalence and univariate analyses for vulvovaginal candidiasis among 221 patients. Statistically significant association was identified between the age group ≤ 40 years ($p < 0.0001$; Odd ratio (OR) = 56.4), previous antibiotics users ($p < 0.0001$; OR = 4.37), previous azole users ($p = 0.0054$; OR = 2.29), AIDS patients ($p =$

0.0003; OR = 3.74), HAART (patients those do not received) ($p = 0.0002$; OR = 0.06), CD4 count < 200 cell/mm³ ($p = 0.0023$; OR = 3.08), working women ($p = 0.0001$; OR = 62.13) and patients belongs to poor economic status ($p = 0.0003$) with VVC. Patients without medical awareness were found more frequently associated with VVC, though without a significant relationship.

Table 3: Proportional prevalence and univariate analysis for vulvovaginal candidiasis among 221 women

Variable	Level	PP	Patients		P value	OR (95% CI)
			with VVC (n=71)	without VVC (n=150)		
Age group	≤ 40 years	61.3	68	43	0.0001	56.4
	> 40 years	2.7	3	107		
Antibiotic	Yes	52.4	43	39	0.0001	4.37
	No	20.1	28	111		
Azole	Yes	42.9	39	52	0.0054	2.29
	No	24.6	32	98		
AIDS diagnosed	Yes	57.5	23	17	0.0003	3.74
	No	26.5	48	133		
HAART	Yes	4.5	1	21	0.0029	0.08
	No	35.2	70	129		
CD4 count cells/mm ³	< 200	53.8	21	18	0.0023	3.08
	200-500	27.5	50	132		
Medical awareness	Yes	27.6	27	71	0.2460	0.68
	No	35.8	44	79		
Occupation	Working	84.3	59	11	0.0001	62.13
	Housewives	7.9	12	139		
Socio economic status	Upper class	0	0	10	0.0003 ^C	Nd
	Middle class	20.0	16	64		
	Poor class	41.9	55	76		

PP, proportional prevalence; VVC, vulvovaginal candidiasis; HAART, highly active anti-retroviral therapy; F, Fisher's Exact Test; C, Chi square test; OR, odd ratio; CI, 95% confidence interval, Nd, not detected; p value of < 0.05 was considered statistically significant.

In the present study, 52.3% (23) of 44 Group 1 patients and 33.6% (48) of 143 Group 2 patients with pruritus vaginas were *Candida* culture positive. Group 1 patients with complaints of burning during micturition had higher 42.4% (14/33) *Candida* culture positive than Group 2 patients 23.6% (21/89). There was no significant difference in *Candida* carriage

was observed in patients with low back pain and lower abdomen pain (Figure 1). Group 1 patients having vaginal secretions like white color, malodorous with curdy character and thick consistency showed higher *Candida* culture positive than Group 2 patients (Figure 2).

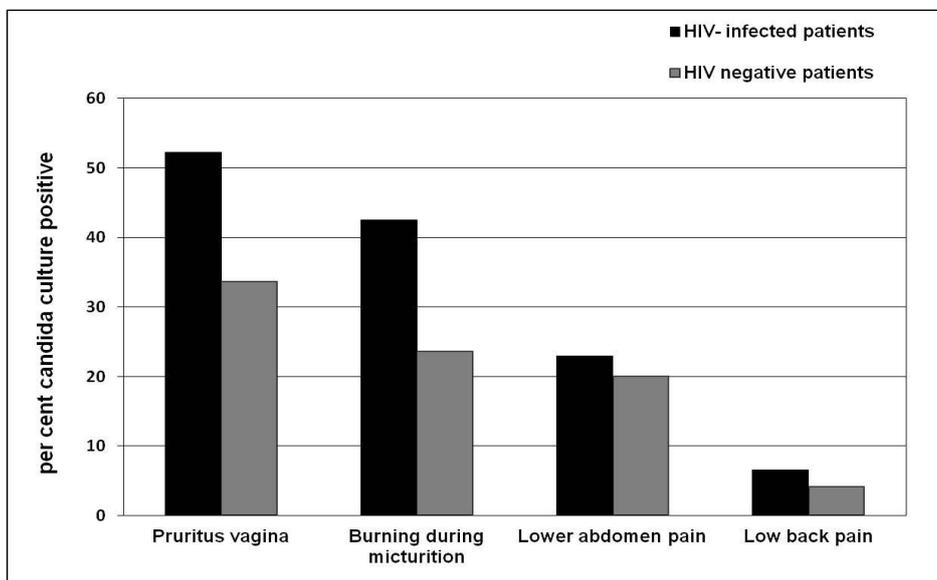


Fig 1: Symptoms of vulvovaginal candidiasis affecting candida culture positivity

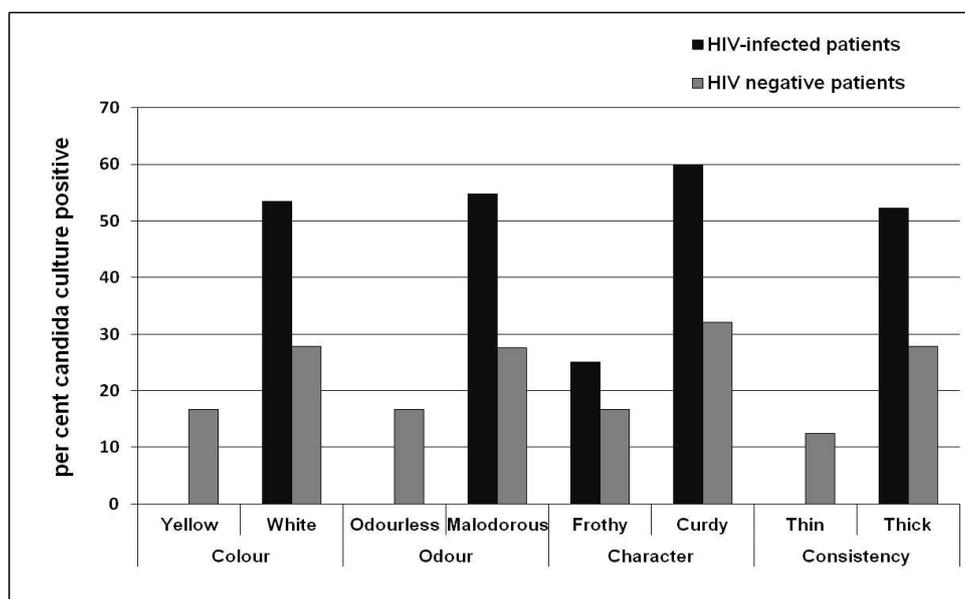


Fig 2: Characteristics of vaginal discharge affecting candida culture positivity

DISCUSSION

In the present study, direct microscopic examination revealed 92.9% (66/71) were positive for yeast cells and mycelia. A previous study reported that 65-85% sensitivity of microscopic examination and found it very sensitive, valuable and simple method for the diagnosis of VVC [6]. The management of vaginal discharge is syndromic and empirical, it is usually

based on naked eye examination of vaginal discharge and that is unsatisfactory because the diagnostic accuracy is lost without microscopic examination [10].

In this study, prevalence of 52.3% (23) *Candida* among HIV-infected group is in agreement with previous studies [9, 11, 12] those reported 66.3% to 72.3% yeast carriage in HIV positive patients. C.

albicans is still recognized as the most frequent aetiologic agent of VVC and RVVC, with a highly significant relationship between high estrogen levels and the occurrence of infection, probably due to the production of glycogen, an attractive substrate for the yeast, by oestrogen stimulated epithelial cells [13]. In the present study, prevalence of *Candida* culture positivity was 27.1% to 52.3% in HIV negative and HIV-infected patients respectively, which is in agreement with studies from India [6,14] and elsewhere [15,16] with rate ranging from 20.8 to 37.4%. The chi square independence test ($\chi^2=15.90$; $P=0.0004$) showed that there is a significant association of *Candida* infection and HIV positive status. Among all, 69.7% (53) of 76 yeast isolates were *C. albicans*. The remainder were NAC species, the most common of which was *Candida glabrata*. Several reports indicate the predominance of the species *C. albicans* in VVC episodes; however, other species appear to be increasing associated with recurring episodes [16]. When the prevalence of vaginal NAC isolates among all HIV-infected and seronegative women were considered, infected and seronegative women were equally likely to be colonized with NAC species. Prevalence of NAC isolates were 31.4% and 28% in HIV negative and HIV-infected group respectively this is in agreement with previous studies [15] those reported 17 to 24% NAC species vaginitis. In a study, Mohanty [8] reported 64.8% carriage of NAC species. *Candida glabrata* is the primary NAC species emerging in vulvovaginal candidiasis, accounting for up to 14% of infections in immune-competent women [2]. In many parts of the world, NAC species isolates notably *C. glabrata* affect 10-20% of women [17]. Vaginitis induced by NAC species is clinically indistinguishable from that caused by *C. albicans*; moreover, such species are often more resistant to treatment [15]. Two HIV infected patients and 3 HIV negative patients were found to carry more than one *Candida* species in their vaginal secretions. The presence of more than one species of *Candida* in the vaginal secretion may predispose to recurrent VVC mainly with species resistance to azole drugs such as *C. glabrata* and *C. krusei*.

Our study clearly reveals that vulvovaginal candidiasis (VVC) was higher in sexually active women between age group 18 to 40 years. It is well known that women with age group of 18 to 40 years have active menstrual cycle therefore they are more prone to *Candida* infection [8]. High level of reproductive hormones present during this stage might be providing an excellent carbon source for the growth of *Candida* by providing higher glycogen content in the vaginal tissue [6]. Mohanty [8] reported 18.5% positive cases in 111 married and sexually active women between age group 18-49 years.

All the patients in our study belonged to rural area of Meerut and Muzaffarnagar, India therefore medical awareness and socio-economic status are also important factors, determining the prevalence of VVC. In our study, chi square independence test showed that there is significant association between with patients belongs to poor class and *Candida* culture positivity ($\chi^2=15.97$; $P=0.0003$). These people are unable to afford the cost of proper diagnosis. The confirmatory diagnosis based in culture is not routinely performed nor generally advised in these regions, because all procedures are expensive and time consuming. Graduates and above educated women were considered as medically aware. Patients without medical awareness were more frequently present in association with VVC, though without a significant relationship. Working women showed higher *Candida* culture positive than housewives. It may possibly be because of greater exposure of working women to the unhygienic condition in working environment. The lesser rate in housewives could be because of their stay in protective environment in house.

In this study, proportional prevalence of VVC was found higher in patients who had previously exposed to antibiotics and or azole in the past. Antibiotics are thought to predispose women to VVC by eliminating the protective bacterial flora, thus allowing *Candida* overgrowth in the gastrointestinal tract, vagina, or both [18]. In this study, amongst HIV-seropositive patients 52.3% (23/44) were previously received over-the-counter antifungal treatment without proper diagnosis of VVC. According to some previous studies, the increased use of over-the-counter antifungal drugs and prolonged treatments for recurrent candidiasis are risk factors for the emergence of azole resistance among *C. albicans* isolated from vulvovaginitis patients [19]. We emphasized that before using over-the-counter vaginal antifungal drugs women are advised to consult their health center if it is the first time they have experienced symptoms of thrush or if they have had more than two infections in the last 6 months. Patients with AIDS diagnosed showed significantly higher rates of *Candida* culture positivity than patients without AIDS. Highly active antiretroviral therapy (HAART) has been associated with a dramatic decrease in the rate of HIV related opportunistic infections [20]. In this study, 50.0% (22) of 44 HIV-infected patients had received HAART treatment and amongst them only one was *Candida* culture positive. It is intuitive to expect an increase of VVC in patients with low CD4 T lymphocyte count (< 200 cells/mm³) [21] as is reflected in the current study. In this study, proportionally 53.8% patients with VVC have been classified with CD4 count < 200 cell/mm³.

The most specific symptom in vulvovaginal candidiasis is pruritus, and even this criterion correctly

predicted VVC in only 38% of patients [22]. In our study, pruritus vagina was common in both HIV-infected and HIV negative patients, though significant difference in *Candida* culture positivity was found between both groups. Patients with pruritus vagina and burning during micturition were more prone to *Candida* infection in contrast to patients with other symptoms. HIV-infected women having vaginal secretions like white color, malodorous with curdy character and thick consistency showed higher *Candida* culture positive than HIV negative patients populations with same symptoms. Our findings are in agreement with previous reports. In India, Ahmad [23] from Aligarh Muslim University reported 20.4% *Candida* culture positive in 215 women with most common pruritus vagina and vaginal erythema. In another study, Khan [10] from Islamic International Medical College, Pakistan, reported that thick creamy vaginal discharge and malodour of discharge associated with maximum infectivity (90%) of *C. albicans* infection.

Data from our study suggested that VVC was significantly associated with age, use of antibiotics/azoles, AIDS, CD4 count and HAART (patients who had not received). Our study is limited to rural area of therefore medical awareness; occupation and socio economic status are also important risk factors determining VVC. Although *C. albicans* was frequently isolated species but non-*albicans Candida* species are not uncommon and their incidence is rising in patients with suppressed immune status. Therefore, systematic examination for HIV-infected patients should be recommended together with the analysis of their immune status for better management of opportunistic infections in these patients. Our results are important for the development of strategies to eliminate these indicators of risk and significantly reduce VCC in rural Indian HIV-infected patients.

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DECLARATION OF INTERESTS

The authors report no declarations of interest. The authors alone are responsible for the content and writing of the paper.

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