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Research Article

Microbiological and clinical assessment of *Candida* carriage in different clinical samples from HIV-infected and non infected patients

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Abstract: The aim of this study was to evaluate *Candida* carriage in different clinical samples from HIV-infected and non infected patients. A total of 574 individuals comprised of 180 HIV-infected patients and 394 HIV seronegative individuals with symptoms of candidiasis were included in this study. The samples were collected from the oral lesions, blood, vaginal fluid, urine, nail scrapings, male genital and cerebrospinal fluid of patients. Clinical samples were aseptically inoculated onto Sabouraud's dextrose agar (SDA) for pure culture. Yeast recovered were speciated by standard techniques. Prevalence of OC was 83.3% in HIV-infected patients, while 68.3% in HIV seronegative individuals. *Candida albicans* was the dominant species in both groups, while non-*albicans Candida* (NAC) isolates were 39.9% and 39.4% in HIV-infected patients and HIV seronegative individuals respectively. *C. albicans* was dominant among oral cavity samples of both HIV-infected (65.8%) and HIV negative (67.8%) patients. In contrast, among bloodstream samples NAC species responded for almost 54.8% to 77.4% of both HIV-infected and HIV negative group respectively. The increased in prevalence of non-*albicans Candida* species in HIV seronegative patients could be due to previous use of antibiotic and azole. Although *C. albicans* was frequently isolated species but non-*albicans Candida* species are not uncommon in India and there incidence is rising in patients with suppressed immune status. Therefore systematic oral 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.

Keywords: HIV-infected, HIV negative, *Candida* carriage, *Candida albicans*, non-*albicans Candida*.

INTRODUCTION

Candida can cause a great variety of infections that includes simple mucocutaneous processes and may also provoke severe invasive infections that can involve virtually any organ. The infections may be acute or chronic, localized or systemic. Disseminated candidiasis is frequently life threatening [1]. The emergence of human immunodeficiency virus (HIV) and AIDS has increased the incidence of mucosal candidiasis caused by *Candida* species [2]. Oral candidiasis (OC) is the most common clinical manifestation in HIV patients [3, 4]. Blood stream infections by *Candida* are also increasingly common and often are associated with high mortality rate [5]. Pathogenic *Candida* mainly occurs as opportunistic infections due to altered host conditions of the host, and at these altered conditions, the fungus proliferates faster [1]. The incidence of genitourinary tract infection is much higher in females during adolescence and childbearing years [6]. Although, *Candida albicans* is the most frequent etiologic agent of candidiasis but in recent years many non-*albicans Candida* (NAC) species such as *C. tropicalis*, *C. glabrata*, *C. krusei* and *C. parapsilosis* have emerged as significant pathogens of clinical importance [7]. The advent of new medical therapies and procedures to treat cancer, the increase in invasive medical procedures, the widespread use of broad-spectrum antibiotics, corticosteroids and immunosuppressive therapy have all been linked with this increase in fungal infections [2, 8]. 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. Therefore we decided to conduct this study, in which all steps starting from patient's selection, filling proforma examination of signs and symptoms, HIV status, CD4 count and culture test were carried out to gather the real state of candidiasis in these patients.

Patients

This prospective study was conducted from January 2005 to December 2008. A total of 180 HIV-infected patients and 394 HIV seronegative patients with symptoms of candidiasis were included in this study. These patients were recruited from outpatients department of Lala Lajpat Rai Memorial Medical College and Hospital, Meerut, India. The individuals were clinically examined by physician for the clinical signs and symptoms of candidiasis. Detailed case history was taken from each patient on a case history proforma which included demographic information and presence of systemic co-infection. The data obtained from clinical history of each patient were the following: previous antibiotic and antifungal treatment, CD4 T lymphocyte counts and treatment with highly active antiretroviral therapy (HAART). 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.).

EXPERIMENTAL SECTION

Sample collection and processing

Samples were collected aseptically from oral cavity, blood, vaginal fluid, urine, nail scrapings, male genital scrapings and cerebrospinal fluid of patients.

Oral samples

The patients were asked to rinse the mouth with 10 ml of sterile Phosphate Buffered Saline (PBS, pH 7.2) for one minute and thereafter oral rinse was collected in sterile container. The oral rinse specimen was immediately centrifuged (Sigma 2K 15) at 3000 xg for 10 minute at 4°C. The supernatant was discarded and sediment was resuspended in 1 ml of sterile PBS and vortexed for 1 minute. 100 µl of this preparation was inoculated onto Sabouraud's Dextrose Agar (SDA) (Hi-Media, India) aseptically and was spread evenly with sterile L-spreader. The plates were incubated at 37°C for 48 h. The colonies of *Candida* were counted to assess CFU/ml of rinse sample [9].

Blood samples

Blood samples from 120 patients suspected of candidiasis were collected aseptically into sterile glucose peptone broth and incubated at 37°C for 10 days. Subcultures were made on Blood agar (Hi-Media) and MacConkeys agar (Hi-Media) [10].

Vaginal fluid samples

A total of 110 women belong to age group of ≤ 40 years and > 40 years attended Gynecology and Dermatology department of L.L.R.M. Medical College, Meerut with complaints of vaginal discharge or vaginal itching and irritation were included in this study. Sterile cotton swab was used to collect vaginal fluid. The material on the swab was transferred aseptically onto the SDA supplemented with gentamicin (0.06 µg/ml) and with and without cycloheximide (5 µg/ml). The inoculated plates were incubated for 48 h at 37°C. The pasty, opaque and pale coloured colonies that developed on incubation were subcultured to obtain pure culture [11, 12].

Urine samples

Urine samples of 34 patients were aseptically inoculated on SDA (Hi-Media, India) with antibiotics (chloroamphenicol 20 µg/ml and gentamycin 0.06 µg/ml) and incubated at 37°C for 48 h to obtain discrete colonies and pure cultures of Candida.

Nails scrappings samples

Affected nails scrapings were collected aseptically. A part of sample was incubated in 10% potassium hydroxide (KOH) for 30 minutes and examined directly under a light microscope for fungal elements. The rest of the sample was inoculated onto SDA plates with chloroamphenicol (20 µg/ml) and with and without cycloheximide (5 µg/ml), incubated at 37°C for 72 h [13]. The pathogenic Candida appeared as dry, white and spreading colonies on SDA plates.

Male genital scrapings

Specimens for yeast culture were collected from the glans penis and inner preputial layer using the direct impression on CHROMagar Candida medium and by swabbing with a sterile cotton swab.

Cerebrospinal fluid

5 ml of cerebrospinal fluid sample was collected and centrifuged at 2000 xg for 10 minutes at 4°C. Without disturbing the sediment, supernatant was aseptically removed with a sterile pipette. Resuspend in 2 ml PBS and vortexed. Aseptically inoculated onto SDA plates with antibiotics (chloroamphenicol 20 µg/ml and gentamicin 0.06 µg/ml) and incubated at 37°C for 48 h to obtain discrete colonies and pure cultures.

Strain identification

The yeast isolates were identified according to morphological characteristics and the biochemical profile. Yeast samples were first subjected to germ tube production test. To determine yeast micromorphology, cornmeal-Tween 80 agar plates were streaked and stabbed with a 48-h-old yeast colony, covered with a sterile coverslip, incubated at room temperature for 3 to 5 days in dark to promote the production of chlamydospores, hyphae, pseudohyphae, and arthroconidia. Biochemical tests were performed by assimilation method using HiCandida KB006 Kit (Hi-Media) 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 ($2.5x10^3$ CFU/ml), and incubated at 25°C for 24-48 h. Change in colour indicates assimilation of the respective carbohydrate sources. Fermentation of sugars was performed by inoculating 100µl ($2.5x10^3$ CFU/ml) of 48-h culture suspensions of test isolates into tubes of fermentation broth containing 2% solutions of the respective sugars. A positive result was indicated by production of acid and gas [14].

RESULTS

The patient's demographics are summarized in Table 1. Out of 180 HIV-infected patients 83.3% (150) were candida culture positive, while 68.3% (269) of 394 HIV seronegative individuals showed candida carriage. *Candida albicans* was the most common isolate from both groups. Non-*albicans Candida* (NAC) isolates were 39.9% (61) and 39.4% (106), in HIV-infected patients and HIV seronegative individuals respectively.

Table 1: Demographics of patients.

Demographics	**HIV-infected patients**	**HIV seronegative individuals**
No. of patients	180	394
Gender		
Male	165 (91.7)	247 (62.7)
Female	15 (8.3)	147 (37.3)
Mean Age (range years)	34.6 (25-64)	40.2 (32-62)
Saliva flow rate		
reduced	121 (67.2)	178 (45.2)
normal	59 (32.8)	216 (54.8)
CD4 count, cells/mm^3		
0-200	167 (92.8)	
201-500	17 (9.4)	43 (10.9)
>500	-	351 (89.1)
Antibiotic received	77 (42.8)	213 (54.1)
Azole received	91 (50.6)	108 (27.4)
AIDS diagnosed	117 (65)	-
HAART received	42 (23.3)	-
Gutaka chewer (ST)	165 (91.7)	300 (76.1)
Alcohol	73 (40.6)	147 (37.3)

Data are shown in no. (%) of patients, unless otherwise indicated; ST=Smokeless tobacco

Table 2: Specimen wise species distribution and *Candida* carriage in HIV-infected patients.

Species	Specimen type			Total (n=150)
	Oral (n=114)	Blood (n=31)	Vaginal fluid (n=5)	
C. albicans	75 (65.8)	14 (45.2)	-	89 (59.3)
C. tropicalis	14 (12.3)	6 (19.4)	-	20 (13.3)
C. glabrata	8 (7.0)	1 (3.2)	5 (100)	14 (9.3)
C. parapsilosis	5 (4.4)	2 (6.5)	-	7 (4.7)
C. krusei	4 (3.5)	2 (6.5)	-	6 (4.0)
C. dubliniensis	3 (2.6)	2 (6.5)	-	5 (3.3)
C. famata	2 (1.8)	2 (6.5)	-	4 (2.7)
C. guilliermondii	3 (2.6)	-	-	3 (2.0)
C. kefyr	-	1 (3.2)	-	1 (0.7)
C. haemulonii	-	1 (3.2)	-	1 (0.7)
NACs	39 (34.2)	17 (54.8)	5 (100)	61 (39.9)

Data are shown in no. (%) of isolates, unless otherwise indicated; NACs=non-*albicans Candida* species

Out of 122 oral cavity samples from HIV-infected patients, 114 yeast representing 8 *Candida* species were isolated. *C. albicans* (65.8%) was the dominant species, while NAC species isolates constituted 34.2% (39/114). Among 39 NAC isolates, *C. tropicalis* (12.3%) was the leading species followed by *C. glabrata* (7.0%), *C. parapsilosis* (4.4%), *C. krusei* (3.5%), *C. dubliniensis* and *C. guilliermondii* (2.6% each) and *C. famata* (1.8%) (Table 2). Out of 178 oral cavity samples from HIV seronegative individuals, 164 yeast representing 8 *Candida* species were isolated. *C. albicans* (67.1%) (110) was the dominant species and NAC species isolates were 32.9% (Table 2). Among NAC isolates, *C. tropicalis* accounted for 14.6% followed by *C. glabrata* (5.5%), *C. parapsilosis* (4.9%), *C. krusei* (3.7%), *C. dubliniensis* (1.8%) and only two isolates of *C. famata* and *C. guilliermondii* each (Table 3).

A total of 53 blood samples from HIV-infected were evaluated. Among them 31 yeast representing 9 *Candida* species were isolated, of which *C. albicans* accounted for 45.2% isolates and it was the single most prevalent species, however NAC isolates collectively constituted 54.8%, included *C. tropicalis* (19.4%) as the leading species followed by *C.*

parapsilosis, *C. krusei*, *C. dubliniensis* and *C. famata* (6.5% each) and only one isolate of *C. glabrata*, *C. kefyr* and *C. haemulonii* (Table 2). Among HIV seronegative individuals a total of 22.6% isolates of *C. albicans* were recovered, while NAC isolates collectively constituted 77.4% isolates, included *C. glabrata* (16.1%) as leading species followed by *C. tropicalis*, *C. parapsilosis* and *C. kefyr* (12.9% each), *C. krusei* (9.7%), *C. famata* (6.5%) and only one isolate of *C. glabrata*, *C. dubliniensis* and *C. haemulonii* (Table 3).

All five vaginal fluid samples from HIV-infected patients yielded only 5 *C. glabrata* isolates (Table 2), while 52 isolates were recovered from HIV negative individuals. Among them *C. albicans* (67.3%) was the single most frequent species, while NAC collectively constituted 32.7% isolates which included *C. glabrata* (17.3%), followed by *C. tropicalis* (11.5%) and two isolates of *C. parapsilosis* (Table 3).

Among 18 urine isolates from HIV negative individuals, *C. albicans* (55.6%) was the dominant species followed by *C. tropicalis* (16.7%), *C. guilliermondii* and *C. lusitaniae* (11.1% each) and only one isolate of *C. glabrata*. A total of 7 samples of nails scrapings were evaluated of which only two isolates of *C. krusei* was isolated. One isolate of *C. albicans* and *C. viswanathii* each was isolated from male genital and cerebrospinal fluid samples respectively (Table 3).

Table 3: Specimen wise species distribution and *Candida* carriage in HIV seronegative individuals

Species	Specimen type							
	Oral (n=164)	Blood (n=31)	Vaginal (n=52)	Urine (n=18)	NS (n=2)	MG (n=1)	CSF (n=1)	Total (n=264)
C. albicans	110 (67.1)	7 (22.6)	35 (67.3)	10 (55.6)	-	1 (100)	-	163 (60.6)
C. tropicalis	24 (14.6)	4 (12.9)	6 (11.5)	3 (16.7)	-	-	-	37 (13.8)
C. glabrata	9 (5.5)	5 (16.1)	9 (17.3)	1 (5.6)	-	-	-	24 (8.9)
C. parapsilosis	8 (4.9)	4 (12.9)	2 (3.8)	-	-	-	-	14 (5.2)
C. krusei	6 (3.7)	3 (9.7)	-	-	2 (100)	-	-	11 (4.1)
C. dubliniensis	3 (1.8)	1 (3.2)	-	-	-	-	-	4 (1.5)
C. famata	2 (1.2)	2 (6.5)	-	-	-	-	-	4 (1.5)
C. guilliermondii	2 (1.2)	-	-	2 (11.1)	-	-	-	4 (1.5)
C. kefyr	-	4 (12.9)	-	-	-	-	-	4 (1.5)
C. lusitaniae	-	-	-	2 (11.1)	-	-	-	2 (0.7)
C. haemulonii	-	1 (3.2)	-	-	-	-	-	1 (0.4)
C. viswanathii	-	-	-	-	-	-	1 (100)	1 (0.4)
NACs	54 (32.7)	24 (77.4)	17 (32.7)	8 (44.4)	2 (100)	-	1 (100)	106 (39.4)

Data are shown in no. (%) of isolates, unless otherwise indicated; NACs=non-*albicans Candida* species; NS= Nail scrapings; MG= Mail genital; CSF=Cerebrospinal fluid.

DISCUSSION

Number of opportunistic fungal infections has been increasing in HIV-infected patients. In past few decades, there have been numerous reports of *Candida* infections in India [5, 8-9, 15-16]. The predominance of *C. albicans* (60.1%) and non-*albicans Candida* (NAC) species isolates (39.9%) in the present study is in agreement with previous reports in India [5, 15] and abroad [17-18]. In a study, Arora *et al.* [5] in tertiary care hospital, Faridkot, Punjab reported *C. albicans* (78%) as the dominant species followed by *C. parapsilosis* (13%) and *C. tropicalis* (9%). In another study, Sengupta and Ohri [15] reported that out of 63 isolates of yeast, *C. albicans* (66.6%) was dominated followed by *C. tropicalis* (14.3%), *C. parapsilosis* (6.3%), *C. kefyr* (3.2%), *C. krusei* (3.2%) and *C. guilliermondii* (1.6%). Sandven [17] reviewed 24 studies addressing episodes of candidiasis in United States tertiary care hospitals and observed that the incidence of *C. albicans* isolates ranged from 38.8% to 79.4% of all episodes. Wang *et al.* [18] from National Taiwan University Hospital, Taiwan reported that among 230 blood and 344 non blood isolates *C. albicans* (49.1%) was leading species followed by *C. tropicalis* (21.7%), *C. parapsilosis* (13.9%) and *C. glabrata* (13%) are confirmed by our studies in India. It may therefore, be concluded that *C. albicans* is the most dominated opportunistic pathogenic species not only India but throughout the world and roughly estimate more than half of total sum of all species.

Oral candidiasis (OC) is the most frequent opportunistic fungal infection among HIV- infected patients, and it has been estimated that more than 90% of HIV-infected patients develop OC often debilitating infection at some time during progression of their disease [19]. Present study clearly reveals that *C. albicans* is the most prevalent species in oral cavity of both HIV-infected and HIV seronegative population, which confirm that *C. albicans* is the most frequent etiologic agent of oral candidiasis in India. A study from SDM College of Medical Sciences, Karnataka, India reported that HIV-infected patients with oral candidiasis showed 97.3% of *Candida* isolates [9] in

another study from Andhra Medical College, India reported 77.8% isolates of *Candida* species, among 54 single isolates from sputum of HIV-infected patients [16]. Mucocutaneous candidiasis is probably one of the commonest manifestations of HIV-infected status worldwide with OC being most widely reported. In India, its incidence has been reported from 50 to 100%. Type of lesions may vary and some of the patients may lack classical picture of oral thrush especially when CD4 count is quite high [20]. *Candida* infections, with oral thrush and esophagitis as frequent clinical manifestations, are the most common opportunistic infections encountered in AIDS patients [21].

There is significant geographic variation as observed among cases of candidemia in different part of the world. Coincidently, we found equal number *Candida* carriage in bloodstream of both HIV-infected and HIV negative group (Table 2). Although *C. albicans* was frequently isolated species in bloodstream of both HIV-infected (45.2%) and HIV negative (22.6%) patients but collectively NAC species incidence rate was higher in both groups. In bloodstream infection NAC species responded for almost 54.8% to 77.4% of both HIV-infected and HIV negative group respectively, while in oral cavity *C. albicans* was the dominant species. The increased in prevalence of non-*albicans Candida* species in both groups could be due to previous use of antibiotic and azole. *C. tropicalis* fungemia was the second most common spp. of *Candida*. Epidemiological data from the Indian subcontinent showed that 67 to 90% of nosocomial candidemia cases were due to NAC species of which *C. tropicalis* was the most dominant [7, 22-25]. *C. famata* and *C. dubliniensis* are very rarely isolated from blood samples causing candidemia. There are numerous reports from India and abroad in last few decades that supports our findings. In a study from Taiwan reported nosocomial candidemia with high percentage 20 to 21% of *C. tropicalis*, with this organism ranking second among all species of *Candida* isolates [26]. In another study of candidemia conducted in United States hospitals, *C. glabrata* accounted for 10% to 21 % of all candidemic episodes which is similar to our findings [27-28]. Basu *et al.* [29] reported, 65% *Candida* isolates from candidemia patients with HIV infection and 35% *Candida* isolates from neonates in Peerless Hospital and BK Roy Research Centre, Kolkata, India. Colonization of *C. tropicalis* also had higher predictive value of subsequent invasive candidiasis than other *Candida* species [30].

In the present study, *C. glabrata* was the only species isolated from vaginal fluid of HIV-infected patients and it was dominated among NAC isolates of HIV negative patients. It suggests that *C. glabrata* is the most common etiologic agent of vulvovaginal candidiasis. The overall prevalence of vulvovaginal candidiasis was found 13.6% which is comparable to other studies from India [31-32] and elsewhere [1, 33] with rate ranging from 20.8 to 37.4%. In the present study, prevalence of NAC species vaginitis was found 32.7% to 100% in HIV negative and HIV-infected group respectively which is in agreement with previous studies [1, 12, 34]. In a study, Akortha *et al.* [1] reported 83.8% isolates of *C. glabrata* from high vaginal fluid swab and in another study, Mohanty *et al.* [12] reported 64.8% carriage of NAC species and *C. glabrata* (50.4%) was isolated as dominant species among 111 isolates. Ray *et al.* [34] reported 61.3% isolates of *C. glabrata* as most common organism among 111 vulvovaginal candidiasis patients. The NAC species vaginitis in this study was relatively higher (38.6%) than some other studies those reported 17 to 24% [33, 35]. The rise in vulvovaginal candidiasis, more specifically in those caused by non-*albicans* species, could be due to several factors, ranging from an increase in use of over-the-counter antifungal to an increase in high-risk patient populations. *C. glabrata* is the primary non-*albicans Candida* species emerging in vulvovaginal candidiasis, accounting for up to 14% of infections in immune-competent women [36]. It may safely be concluded from our studies that *C. glabrata* is the most common etiologic agent for vulvovaginal candidiasis.

Candida carriage in urine samples is comparable (4.3%) to other studies reported 3.7% to 17.1% [1, 37]. Among urine isolates *C. albicans* was most frequent species (55.6%), followed by *C. tropicalis* (16.7%). Isolation of *C. tropicalis* from urine samples is more often indicative of disseminated candidiasis than the isolation of *C. albicans* [38]. *Candida* species particularly *C. albicans* prevails in fingernail infections [39]. In recent years infection due to *C. krusei* has been found increasingly both in immunocompromised and non immunocompromised hosts [40]. The most common cause of balanitis is *C. albicans.* In the present study only one isolate of *C. albicans* was isolated from male genital glans, while another study reported 47 isolates from male genital glans [41].

CONCLUSION

Present study reveals that *C. albicans* is the most dominant species in both HIV-infected and HIV seronegative individuals. Although, *C. albicans* was frequently isolated species but non-*albicans Candida* species are not uncommon in India and there incidence is rising. Amongst non-*albicans Candida* species *C. tropicalis* was the most prevalent species except in vaginal fluid samples. The emergence of *C. glabrata* as a common etiologic agent of vulvovaginitis has important clinical implication due to its innate resistance to azoles. Non-*albicans Candida* species are dominated among bloodstream infection of both HIV-infected and HIV seronegative patients.

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Declaration of interest

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

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