### **Original Research Article**

DOI: https://dx.doi.org/10.18203/2394-6040.ijcmph20205153

# Epidemiological profile of candidiasis in HIV/AIDS patients in a tertiary care hospital

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Received: 13 July 2020 Revised: 12 October 2020 Accepted: 13 October 2020

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#### **ABSTRACT**

**Background:** Clinical research in fungal infections is largely a neglected area in health care settings in India. Candida species cause diseases ranging from mucosal infections to systemic mycoses depending on host's immune status. Aim of this study was to evaluate the clinico-epidemiological profile of candidiasis, and their correlation with an immunological profile in HIV/AIDS patients.

**Methods:** Clinical details and investigations of 200 symptomatic, confirmed HIV-positive patients, suspected of having candidiasis were recorded and analyzed. Relevant clinical samples depending on the organ system involved were collected and subjected to direct microscopy, culture isolation and serology. Identification and speciation of the isolates was done by biochemical methods as per standard recommended procedures. CD4-count was determined by flow cytometry using Fluorescent Activated Cell Sorter Count system.

**Results:** Patients ranged from 17-65 years with a mean age of 33.83±9.07 years. Most common clinical presentations were seen to be white oral patches (82%), weight loss (79%), fever (67%), loss of appetite (53%), headache (51.5%), cough (38.5%) and diarrhea (30%). Gastrointestinal system (35%) was the most commonly involved system. The CD4-counts ranged from 16-1033 cells/µl. 93 (46.5%) patients had CD4-counts <200 cells/µl, while CD4-count <100 cells/µl was seen in 40 (20%) and CD4-count <50 cells/µl in 20 (10%). Candidiasis was detected in 60% of the patients. Yeasts isolated were *C. albicans* (82.51%), *C. tropicalis* (6.29%), *C. krusei* (4.89%), *C. parapsilosis* (3.49%), and *C. glabrata* (2.79%).

**Conclusions:** *C. albicans* was predominant species and presence of oral candidiasis is a matter of concern. Early and accurate diagnosis of candidiasis is one of the keys helps for the success of effective HIV/AIDS disease management.

Keywords: HIV, Candidiasis, Epidemiology, AIDS, CD4 - count

#### INTRODUCTION

In the last decade, a change in the epidemiology of fungal infections has been observed and with an increase in the incidence of fungal infections, mainly due to rising numbers of immunocompromised patients, more

advanced treatments and longer hospital stays.<sup>1</sup> Various fungal infections vary from superficial infections to systemic or disseminated disease in HIV/AIDS patients.<sup>2</sup> World health organization (WHO) predictions put India as one of the biggest repository of HIV/AIDS patients in coming decades.<sup>3</sup>

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Oropharyngeal candidiasis (OPC), which is caused by Candida species, is a common oral opportunistic infection among human immunodeficiency virus (HIV) positive individuals, with occurrence rates of 50-95%.<sup>4</sup> Oropharyngeal Candidiasis tops the list of opportunistic infections in HIV/AIDS patients and represents the most common clinical presentation of mucosal candidiasis associated with HIV/AIDS.<sup>5</sup>

*C. albicans*, the primary causative agent, is a commensal organism of the gastrointestinal and reproductive tracts.<sup>6</sup> It is an important pathogen, especially in immunocompromised patients.<sup>5</sup> In individuals who experience immunosuppression by HIV infection or undergo immunosuppressive therapy, *C. albicans* can become pathogenic, resulting in symptomatic infection. In HIV positive individuals, the incidence of OPC correlates with reduced systemic CD4<sup>+</sup>T cell counts, which indicates that systemic T cells have a role in protecting against OPC.<sup>7</sup>

Candida spp. is now considered fourth in the rank of the causative agents of nosocomial invasive infection with a high mortality and morbidity. The incidence of opportunistic fungal infections is increasing with the ever-changing spectrum of fungal disease and selection of fungi. Resistance of fungi to the few commonly available antifungal drugs is on the rise and cross resistance is being increasingly recognized. An early specific diagnosis and subsequent treatment to combat this infection is not only the concern of western hospitals but is also equally relevant to developing countries like India.

The clinico-epidemiological data from India on the spectrum of candidiasis in HIV/AIDS patients, and immunological profile of these patients are scarce. Hence, the aim of this study was to evaluate the clinico-epidemiological profile of candidiasis and their correlation with the immunological profile in HIV/AIDS patients.

#### **METHODS**

#### Period of study

This study was implemented at Maulana Azad Medical College and Associated Lok Nayak Hospitals, New Delhi from March 2012 to February 2015, to evaluate the clinico-epidemiological profile of candidiasis and their correlation with the immunological profile in HIV/AIDS patients.

#### Study design

Prospective observational study.

#### Study population

Two hundred symptomatic confirmed HIV-positive adult patients, of both sexes, suspected of having a fungal infection were taken as subjects. Cases were recruited from the outpatient department, wards and the Anti-Retroviral Treatment Clinic of the hospital. All patients were evaluated by a pre-designed protocol covering the biodata, history, including high-risk behavior, mode of transmission, marital status, partner status, presenting complaints and physical examination. Depending on the clinical symptoms, relevant clinical samples were collected with complete universal precautions.

Inclusion and exclusion criteria of samples: symptomatic confirmed HIV-positive adult patients, of both sexes, suspected of having a fungal infection were included in the study. Relevant clinical samples from these patients depending on the organ system involved like oropharyngeal swab, sputum, blood, urine, CSF, stool was collected.

#### Ethical approval

Ethical approval was granted by the institutional ethical committee of the College & Associated Hospitals, India. Patients provided written consent and they were informed that their participation was voluntary and that they could withdraw from the study at any stage without incurring any penalty.

The immune status was assessed by performing the CD4 count of each patient enrolled in the study, by flow cytometry using the fluorescence activated cell sorter BD FACS count system (becton dickinson) as per the manufacturer's instructions.

#### Microscopy, culture and identification

Relevant clinical samples depending on the organ system involved like oropharyngeal swab, sputum, blood, urine, CSF, stool was collected and all other than blood sample were subjected to direct microscopy using gram staining and KOH wet mount. Culture was done on sabouraud dextrose agar, with and without chloramphenicol (16µg/ml). Specimens were streaked in duplicate; one set of inoculated slants was incubated at 25°C and the other at 37°C, and were examined on alternate day for growth up to 4-6 weeks before discarding as negative. The identification and speciation of the isolates was conducted by colony morphology, gram staining, germ tube formation, growth on corn meal agar with tween 80, hi crome candida morphology agar and an enzymatic triphenyl tetrazolium chloride reduction test as per standard procedures.<sup>9</sup> For further characterization, each isolate was subjected to carbohydrate assimilation tests and carbohydrate fermentation tests as per standard recommended procedures. 10-12

#### Statistical analysis

Statistical analysis was performed by SPSS software (version 17.0; SPSS S.L., Madrid, Spain). For all

statistical tests, p<0.05 was considered to indicate a significant difference.

#### **RESULTS**

Two hundred HIV/AIDS symptomatic patients were studied. 155 (79%) patients belonged to the age group 21-40 years, with maximum number belonging to 26-40 years, the most reproductive age group of the country. The M:F ratio in our study was 2.3:1. Clinical and demographic profiles of HIV/AIDS patients with and without Candidiasis are shown in (Table 1). Candidiasis was detected in 60% of the patients.

Table 1: Demographic profile of HIV/AIDS patients with and without candidiasis (N=200).

Characteristics	With candidiasis (120) No. of patient	Without candidiasis (80) its (%)	P value
Sex			
Male (136)	84	52	-
Female (59)	30	29	-
Intersex (5)	5	0	-
Age (mean)	33.8	33.4	0.08
Marital status			
Married (164)	87	77	0.000
Not married (36)	33	3	0.000
Partner status (n=165)			
HIV Positive (96)	50	46	0.04
HIV Negative (32)	17	15	-
Unknown (37)	19	18	-
Mode of HIV infection			
Sexual (143)	81	62	-
Blood and products (12)	8	4	-
Inj. drug user (28)	20	8	-
Homosexual (5)	4	1	-
Not specified (12)	7	5	-
CD4 count			
<200 cells/mm <sup>3</sup> (93)	55	38	-
>200 cells/mm <sup>3</sup> (107)	57	50	-
Total lymphocyte count<1200 cell/ul (33)	28	5	0.002
Tuberculosis (100)	61	39	-

The clinical presenting complaints and organ system involvement in our study population are shown in (Table 2). The most common clinical presenting complaints were seen to be white oral patches (82%), weight loss (79%), fever (67%) and loss of appetite (53%). 75 (37.5%) patients had HB<10gm%, 17 (8.5%) had total leucocytes count<4000cell/ul, with lymphocyte count<1200, and neutrophil count<2000 in 33 (16.5%), 13 (6.5%) patients respectively. In our study, it was seen that the

gastrointestinal system (35%) was the most commonly involved system.

The CD4-counts ranged from 16-1033cells/µl. 93 (46.5%) patients had CD4-counts<200cells/µl, while CD4-count<100cells/µl was seen in 40 (20%) and CD4-count<50cells/µl in 20 (10%) patients depicting a major population with severe immunosuppression (Figure 1).

Table 2: Depicting clinical profile and organ involvement in the patients recruited for the study.

Clinical presentation	Patient (n)	Patient (%)
White oral patches	164	82
Weight Loss	158	79
Fever	134	67
Loss of appetite	106	53
Headache	103	51.5
Cough	77	38.5
Diarrhoea	60	30
Dyspnoea	52	26
Night sweats	32	16
Painful swallowing	32	16
Altered sensorium	27	13.5
Lymphadenopathy	22	11
<b>Burning micturition</b>	19	9.5
System involvement	Patient (n)	Patient (%)
GIT	100	50
Multiple systems	45	22.5
Respiratory system	35	17.5
Central nervous system	13	6.5
Genitourinary system	5	2.5
Skin	2	1

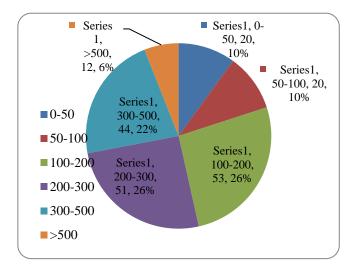


Figure 1: CD4 profile among patients recruited for the study.

A total of 314 samples comprising of oral swabs collected from all patients while sputum, blood, CSF, stool and

urine were collected from 39 (12.4%), 32 (10.2%), 23 (7.32%), 11 (3.50%) and 09 (2.87%) patients respectively depending on the organ system involved. Yeasts were isolated in 120 (60%) of the patients and among these patients Candida species were isolated in 143 (45.54%)

samples. *C. albicans* (82.5%) was the commonest *Candida spp.* isolated, followed by *C. tropicalis* (6.29%) and *C. krusei* (4.89%), *C. parapsilosis* (3.49%) and *C. glabrata* (2.79%) as shown in (Table 3).

Yeast species	No (%)	Oral swabs (No %)	Sputum No (%)	Urine No (%)	Blood No (%)	Stool No (%)
C. albicans	118 (82.5)	98 (83)	18 (15)	0	2 (1.69)	0
C. tropicalis	9 (6.29)	5 (55)	2 (22)	1 (11)	1 (11)	0
C. glabrata	4 (2.79)	3 (75)	1 (25)	0	0	0
C. parapsilosis	5 (3.49)	4 (80)	1 (20)	0	0	0
C. krusei	7 (4.89)	3 (42.8)	1 (14.2)	1 (14.2)	1 (14.2)	1 (14.2)
Total	143	113 (79)	23 (16)	2 (1 39)	4 (2.79)	1 ( 69)

Table 3: Correlation of yeast isolation with clinical specimen.

Out of 118 *C. albicans* strains, maximum number were from oral swabs (83%) followed by sputum (15%) and blood (1.69%) while most of *C. tropicalis* were isolated from oral swab (55.5%), followed by sputum (22%), urine (11%) and blood (11%). *C. glabrata* and *C. parapsilosis* were also mostly isolated from oral swab (75%), followed by sputum (25%) whereas *C. krusei* was isolated from oral swab (42.8%) and (14.2%) each of sputum, urine, blood and stool samples respectively.

Total 113 oral Candida species isolates included; 98 *C. albicans* (86.7%), 5 *C. tropicalis* (4.42%), 4 *C. parapsilosis* (3.53%), 3 each of *C. krusei* (2.65%) and *C. glabrata* (2.65%) while 23 sputum candida species isolates included, 18 *C. albicans* (78.2%), 2 *C. tropicalis* (11.1%), 1 each of *C. parapsilosis* (5.5%), *C. krusei* (5.5%) and *C. glabrata* (5.5%). Four Candida species isolates from blood consisted of 2 *C. albicans* (50%) and 1 each of *C. tropicalis* (25%) and *C. krusei* (25%). One each of *C. tropicalis* (50%) and *C. krusei* (50%) were isolated from urine while only a single isolate of *C. krusei* (100%) was recovered from stool samples.

#### **DISCUSSION**

Patients in our study belonged to an age group of 17-65 years with a mean age of 33.83±9.07 years. 79% patients belonged to the age group 21-40 years, signifying the involvement of sexually active and economically productive population. Similar age groups were reflected in studies conducted in India. The male: female ratio in our study was 2.3:1. A similar finding was reflected in a study by Umesh et al 2012. The disparity could be due to the decreased exposure of women in developing countries to health care. Women are less likely to visit an antenatal clinic/testing center if they are older, have high parity, are illiterate, or poor. Need for Programs for increasing the female attendance in the health care centers has been realized very much. 15

Majority (82%) of our patients were married reflecting the epidemiology of the disease in our country. Similar were the findings in a study in Nigeria in 2005 which found majority 71.4% to be married with 3.5% and 2.6% being those separated and widowed respectively and another study from the same country in 2010 reporting 60.3% to be married and 31.8% unmarried. Out of the 164 married, HIV positive patients, 94 (57.32 %) had their partner also positive and 32 (19.51%) had their partners HIV Negative while the HIV status of 38 (23.17%) was not known. While a study by Umesh et al., 201213 reported 62.9% HIV positivity in spouses with 17.29% HIV negativity and unknown status in 20% patients.

Worldwide, the most common mode of transmission is through heterosexual route3. In this study too, the predominant mode of transmission was heterosexual route (71.5%) followed by Injectable (14%), blood transfusion (6%) and homosexual route (2.5%) quite similar to a report, showing heterosexual transmission to be the major mode of transmission (74%) followed by blood transfusion.<sup>17</sup> Anwar et al 2012 also reported, heterosexual route of transmission to be the main route of transmission (78%).<sup>5</sup> Again in a study, the commonest mode of transmission was heterosexual (84.6%) followed by unspecified causes, again highlighting the fact that heterosexual transmission remains the commonest mode of transmission with other sexual practices being uncommon in this part of the world. <sup>18,19</sup>

Many studies have reported that clinical manifestations such as fever, chronic diarrhea, oral patches, Herpes zoster and joint pains can predict progression from HIV infection to AIDS.<sup>7</sup> In our study, 99.5% patients had more than one presenting complaint, similar to a study where all patients (100%) presented with more than one symptom.<sup>20</sup> Most common presentation in our study was oral candidiasis, seen in 82% patients quite in accordance to a study by Malani et al 2001 reported in 71.25% patients while other study reported oral candidiasis to occur in more than 95% of patients and considered it to be an important marker of the disease and its progression.<sup>21,22</sup> In our study weight loss was seen in 79%

and fever in 67% patients while a study13 reported fever in 52.93% and weight loss in 48.81% patients.

Gastrointestinal system (50%) was the most commonly involved system in our study quite in agreement to other studies. The gastrointestinal (GI) tract is the commonest organ system site for fungal opportunistic infections associated with AIDS and can lead to significant morbidity including pain, difficulty in swallowing, diarrhea, and weight loss. 50% to 93% of all patients with HIV disease had marked GI symptoms during the course of their illness. Diarrhoea is known to affect 40-80% HIV infected persons and is associated with high mortality rates in Sub-Saharan Africa. Diarrhoea is sub-Saharan Africa.

In our study, CD4 ranged from 16-1033 cells/ul and the mean CD4-count was 205 cells/ul while a study by Parisa et al 2010 reported that the CD4 range was 32-1371cells/ul.<sup>22</sup> 46.5% of our patients were immunosuppressed and had a CD4-count<200cells/ul, while a study by Anwar et al 2012 reported 77.5% patients with candidiasis to have CD4-counts<200 cells/ul.<sup>5</sup> Severe immunosuppression was seen with a CD4-count<100 cells/ul in 20% of our patients, while an earlier study by Kallol et al 2011 have reported an incidence 39% in the same group.<sup>25</sup>

Correlation between decreased CD4-counts and onset of oropharyngeal candidiasis in HIV/AIDS patients has been documented in several western literatures, however such reports are lacking in Indian hospitals. In our study, 82% patients had oropharyngeal candidiasis & their mean CD4-count was 195 cells/ul, while a study reported the majority of their HIV-positive patients with OPC (85.7%) with a CD4-count \$\leq 200\$ cell/ul. The severity and progress of the HIV/AIDS disease status was assessed by WHO clinical staging. In our study more than 75% of patients were in WHO clinical stage III and IV quite similar to a study, which had more than half of their patients in WHO clinical stages III and IV. 28

AIDS defining illness like tuberculosis was the most common associated disease in our study group (50%) quite similar to a study by Thesus et al 2009 reported Tuberculosis in 33.3% of their patients. Workers from Nepal also found Tuberculosis accounting for 27.3% cases.<sup>29,30</sup>

In our study, *Candida spp.* isolates included, 82.51% *C. albicans*, 6.29% *C. tropicalis*, 4.89% *C. krusei*, 3.49% *C. parapsilosis*, and 2.79% *C. glabrata spp. C. albicans* was also the most frequently observed species in a study in USA.<sup>21</sup> This is a surprising observation compared to the developed world, where non albicans Candida species is a major problem in HIV/AIDS.<sup>31</sup> A study by Parisa et al 2010 also reported the most abundant species isolated from HIV/AIDS patients to be *C. albicans* (50%) followed by *C. glabrata* (21.4%), *C. dubliniensis* (13.3%), *C. krusei* (9.8%), *C. kefyr* (3.1%), *C. parapsilosis* (1.6%), and *C. tropicalis* (0.8%).<sup>22</sup> Workers

from other study reported *C. albicans* species to be predominant in 78.5% patients followed by *C. glabrata* (22.5%) and *C. tropicalis* (14.1%).<sup>28</sup>

In our study, of the 82.5% *C. albicans* maximum numbers (68.5%) were from oral swabs followed by sputum (12.6%) and blood culture (1.4%). *C. albicans* has also been isolated as the most prevalent yeast from oropharyngeal swabs in HIV/AIDS patients in several countries.<sup>32</sup> In our study among *C. glabrata*, 75% were isolated from oral swabs and 25% from sputum whereas a study reported the isolation of *C. glabrata* mainly from blood culture (36%) and urine (29.7%).<sup>33</sup>

*C. parapsilosis* was isolated from oral swabs (80%) and sputum (20%) in our patients and *C. tropicalis* was isolated from oral swabs (55%), sputum (22%), urine (11%), blood (11%) whereas a study by Anwar et al 20125 reported *C. parapsilosis* to be isolated from oral swabs (66%) and skin (33%) and *C. tropicalis* from oral swabs (57%), blood (27%) and stool (14.3%).

In our study, sputum samples yielded *C. albicans* (78.2%), *C. tropicalis* (8.7%), 4.35% each of *C. glabrata*, *C. parapsilosis*, *C. krusei* respectively. Quite in corroboration to our study, Jha et al 2006 reported *C. albicans* (70%) followed by *C. tropicalis* (13.33%), *C. krusei* (10%), 3.33% each of *C. parapsilosis* and *C. stellatoidea* in patients of lower respiratory tract infection.<sup>34</sup>

C. albicans (50%), C. krusei (25%) and C. tropicalis (25%), were isolated from our blood samples while Marukutira et al reported C. albicans as the most common Candida species (45,3%).<sup>35</sup> However, Pahwa et al reported the isolation of C. tropicalis (20.6%), followed by C. parapsilosis (18.9%), C. albicans (17.2%), C. krusei (10.3%), C. glabrata (3.45%) and 29.3% other Candida species.<sup>36</sup>

#### **CONCLUSION**

In this study, *C. albicans* was the predominant species and the presence of oral candidiasis is a matter of concern. Early and accurate diagnosis of the candidiasis is one of the keys helps for success of effective HIV/AIDS disease management enabling the clinician to initiate the most appropriate antifungal agent as early as possible and reduce the morbidity and mortality thereof. Thus, this study is an attempt, to lead to a better clinical management of HIV/AIDS patients.

Funding: No funding sources Conflict of interest: None declared

Ethical approval: The study was approved by the

Institutional Ethics Committee

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Cite this article as: Dhakad MS, Kaur R, Goyal R, Bhalla P, Dewan R. Epidemiological profile of candidiasis in HIV/AIDS patients in a tertiary care hospital. Int J Community Med Public Health 2020;7:4858-64.