

Prevalence of Oropharyngeal Candidiasis in Human Immunodeficiency Virus Infected Patients Attending The Government Hospital

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Article Receive Date: 10th October 2022

Article Publish Date: 2nd November 2022

DOI: <http://doi.one/10.1729/Journal.32002>

ABSTRACT :

Background: HIV patients are prone for getting Candida infection especially in the mucosal area like oral cavity and pharynx. *Candida albicans* is the main yeast causing oral infections. Recurrent infections in the mucosa are unavoidable when HIV patients are put on antibiotics or corticosteroids therapy and smoking itself is a risk factor resulting in transition of commensal phase Candida to pathogenic, though exact reason is not found.

Aim : To isolate, speciate and test for anti-fungal drugs against Candida species.

Methodology: HIV was tested as per the NACO guidelines at our ICTC. Mycological study was conducted using oral swabs smeared from white patch over tongue, palate and gums. Sabouraud dextrose agar was used to culture yeasts. Cream pasty colonies found were subjected for Candida speciation and characterization by standard identification methods. Fluconazole disks (10µg), Voriconazole disks (1µg) was used for anti-fungal susceptibility testing.

Results: Prevalence of OPC is about 69.09%. *Candida albicans* (59 - 77.66%) was the predominant isolate in our study. 27 of 35 isolates were *C. albicans* under 200 cells/mm³ CD4 count. And seven *C. albicans* were isolated among >500 cells/mm³. All Candida species showed resistance to Fluconazole. Voriconazole was sensitive in 82.4% species and resistant in 17.6% of isolates.

Conclusion: *C. albicans* remains the most frequently isolated yeast causing oral thrush. There is changing trends of causative role of non-albicans in causing Oropharyngeal candidiasis (OPC) which are resistant to azole drug fluconazole. ART significantly reduces the occurrence of OPC. Lower CD4 count shows increased colonization of pathological organisms.

Keywords : HIV patients, Oropharyngeal candidiasis, *Candida Albicans*, Non *Candida Albicans*, Antifungal susceptibility, Fluconazole, Voriconazole.

INTRODUCTION:

Human Immunodeficiency virus (HIV), a Lentivirus affects human immune system affecting helper T cells, macrophages and dendritic cells. Decreased CD4 cells hampers cell mediated immunity, thereby attracts many opportunistic infections, leading to AIDS (Acquired Immunodeficiency Syndrome). *Candida albicans* or other Candida species are the colonizer of oral microbiota in two third of the healthy people. Candida species are considered non-pathogenic yeasts among individuals with good immunity^[1] but HIV-positive patients oropharynx will harbor variety of yeasts as compared to HIV-negative patients.^[2]

Oral Candidiasis incidence rate/density is 9.3 per 1000 person months. CD4 count measures cell mediated immunity. It has a strong correlation with immune suppression and progression of disease.^[3] One of the cardinal sign of AIDS infection and is oral candidiasis. Two third of the AIDS patients present with oral thrush.^[4]

Subsequent development of Oropharyngeal Candidiasis (OPC) can be predicted with the load of Candidal colonization and the species related to it.^[5] The majority of Candida infections associated with HIV is caused by *C. albicans*, other species being *C. tropicalis*, *C. krusei*, *C. glabrata*, *C. dubliniensis*, *C. guilliermondii*, *C. parapsolosis*, *C. kefyr*, and *C. pelliculosa*.^[6,7]

HIV is known to be associated with increased colonization of unusual pathogens, altered microbiome can be attributed to mild disease. Anti-retroviral treatment (ART) plays a major role in reversing the CD4 count and maintains the immune cells thereby prolonging the disease progression.^[8] Use of ART improves colonizing microbiome, checks invasion by the yeasts.

Recurrent infections in the mucosa are unavoidable when HIV patients are put on antibiotic or corticosteroid therapy and smoking itself is a risk factor resulting in transition of Candida, from commensal to pathogenic phase, though exact reason is not found.^[9]

Reports have shown that the prolonged antifungal treatment of OPC results in development of drug resistant OPC. Resistant Candida isolates cannot be removed by usual drug regimens. Hence, this present study is aimed to determine the prevalence of oral Candidiasis in HIV seropositive patients and to assess their drug sensitivity pattern.

OBJECTIVES:

1. To isolate and characterize Candida species from Oropharyngeal Candidiasis among HIV seropositive individuals.

2. To perform antifungal susceptibility testing for Candida isolates.
3. To correlate Oropharyngeal Candidiasis with CD4 count.

METHODOLOGY

Type of study: Cross sectional study

Place of study: Department of Microbiology, “K***** ***** ***** *****s”, M*****I year 2018-19.

Inclusion Criteria: All HIV seropositive patients in the age group 17- 60 years with OPC were included in the study.

Exclusion Criteria: HIV- Seropositive patients on antifungal treatment.

Sample collection: After obtaining written informed consent from 76 HIV seropositive patients attending ART center at our hospital were included in the study. The proforma with the patient demographics, chief complaints, route of infection of HIV, predisposing factors for oral candidiasis was collected from each patient. Oral cavity was examined clinically. Suspected cases of Oral Candidiasis, marked by white curdy patch/dischargethat was present over dorsum of tongue, gums, gingiva, buccal mucosa was obtained, using sterile cotton swabs with aseptic precautions. Swabs were sent immediately to microbiology laboratory for mycological procedures.

Laboratory procedures: HIV testing is done by the standard protocol as per NACO guidelines at our ICTC center, which includes pre-test and post-test counseling. After obtaining informed consent the following HIV tests were done - Coombs test, Merrill's test, Tridot test. CD4 count was determined by flow cytometry (FACSCalibur). For mycological study, swabs were put for direct microscopy by Gram staining to look for budding yeast cells and pseudohyphae. Sabouraud dextrose agar was used to obtain fungal colonies.

SDA plates were examined daily for a week before declaring culture negative report. Fungal growth was identified based on colony morphology. Cream pasty colonies obtained were subjected for Candida species characterization by germ tube test and Dalamou-plate culture on corn meal agar at 28°C.^[9,10]

Antifungal testing was done by Disk diffusion method according to CLSI guidelines M100 on Muller Hilton agar plates with 1% glucose and 10µl of methylene blue. Fluconazole disks (10µg), Voriconazole disks (1µg), were obtained from Himedia, India was used for antifungal susceptibility testing.

Statistical analysis: It was described in frequencies and percentages. Results were expressed as Mean, Standard deviation (SD) Chi Square and ANOVA. Graphs, charts and tables were used for result representation.

RESULTS

In our study 76 HIV seropositive patients visiting ART centre were studied. Patients demographic data and clinical profile are shown in table 1. HIV positive individuals in the age group of 17-60 years were affected by oral thrush. Age group in the early forties (42 years) was commonly affected. In our study the number of males were 39 and females were 37. In our study pseudomembranous type of oral candidiasis was the predominant presentation followed by Erythematous and Angular cheilitis. Of the total, 35 patients had CD4 count less than 200 cells/mm³, 8 patients had >500 cells/mm³ and others were between 200 – 500 cells/mm³.

The microscopy of all the samples obtained from clinically suspected cases of OPC were positive for yeast cells and pseudohyphae. The culture tests yielded cream pasty round colonies with curd like odour. Cultures showing > 10⁵ colonies were considered as positive culture. The germ tube test was positive in 59 cultures and showed chlamydo spores on corn meal agar, hence 59 *C. albicans* were identified. The germ tube test was negative in 17 samples. Out of which 16 were identified as *C. tropicalis* and 1 isolate was *C. kefyr*. Table 2 shows distribution of Candida isolates.

Candida species were correlated with CD4 count as follows, 10 non albicans Candida were isolated in patients with <200 cells/mm³ CD4 count, 6 non albicans Candida in 200 cells/mm³ CD4 count and 1 among >500 cells/mm³.

C. albicans (59 / 77.66%) was the predominant isolate in our study. 27 *C. albicans* was isolated in patients with <200 cells/mm³ CD4 count. And 7 *C. albicans* was isolated with >500 cells/mm³ CD4. Correlation of CD4 count with Candida species given in table 3 and Graph 1.

The antifungal testing was done with two azoles derivatives (Fluconazole 10mcg and Voriconazole 1mcg) against 30 randomly picked isolates of Candida species. In our study all Candida species showed resistant to Fluconazole. Voriconazole was sensitive in 82.4% isolates and resistant in 17.6%.

TABLE 1: Demographic data of HIV patients.

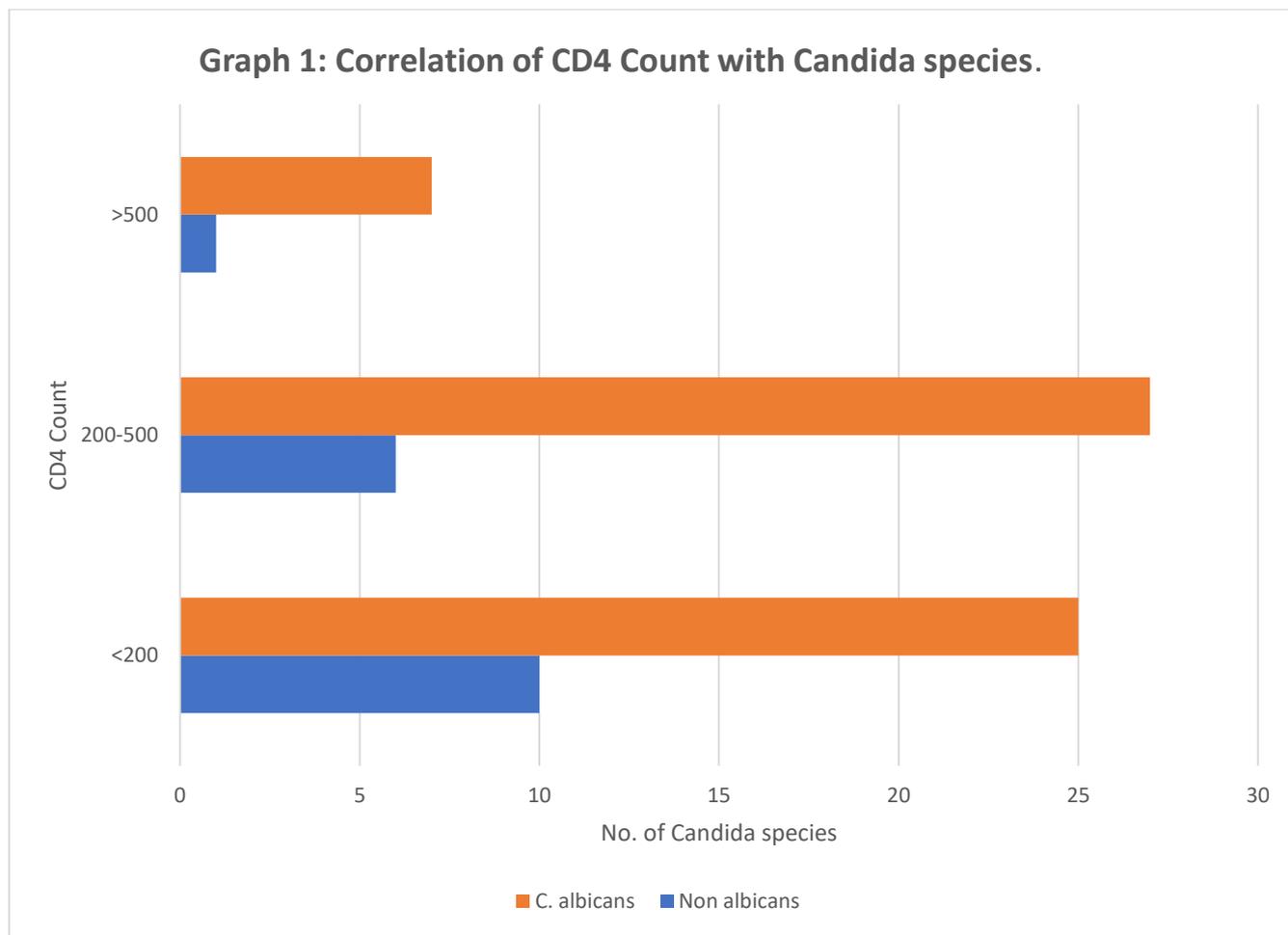
Age in years/ Gender	Male	Female
16 to 30 years	7	7
31 to 45 years	21	20
46 to 60 years	11	10
Total	39	37

Table 2: Isolation of Candida species

	16 to 30 years	31 to 45 years	46 to 60 years	Total n (%)
<i>C. albicans</i>	7	31	22	59(77.66%)
<i>C. tropicalis</i>	4	7	5	16(21.05%)
<i>C. kefyr</i>	0	0	1	1(1.31%)

Table 3: Correlation with CD4 Count

CD4 Count (Cells/mm ³)	<i>C. albicans</i>	<i>C. tropicalis</i>	<i>C. kefyr</i>	Total
<200	25	9	1	35
200-500	27	6	0	33
>500	7	1	0	8
Total	59	16	1	76



DISCUSSION:

Definitive diagnosis and treatment of HIV/AIDS opportunistic infections are of particular concern. Oral thrush can be a mild or overt disease affecting the living of the HIV people. Morbidity due to Candida species has an impact on medical care. Further more, it is known to increase the in-patient days of hospital care. Recurrence with drug resistant candida is well known. Therefore, finding the disease burden and associated determinants is crucial for any health care unit. Various Candida species are the oral microbiome but rarely invades the mucosa. However, immunocompromised patients like HIV infected patients are prone to have overt candidiasis. Candida species prefer moist areas of the body, which is why it is usually found in the oral cavity.

Many other microorganisms grow in healthy mucous membranes in the body. However, when the other organisms become depleted, the yeasts are able to compete for limited resources. Candida species is found to be normal microbiome of the oral cavity of humans. Bad oral hygiene, smoking, tobacco chewing and other social factors lead to variation in oral flora which further increases oral cavity infections. Per se Candida isolation in the oral cavity could hint us about eventual development of oral thrush, hence good oral hygiene using local antiseptics may be recommended at this stage.

The prevalence rate of the disease is 70% among HIV individuals. Totally 110 samples were collected out of which 76 samples showed candida colonies. In our study, the prevalence rate is 69.09%. HIV infection is seen between the age of 17-60 years, thereasons may be, active involvement in sexual activity and alteration in local immunity. And it is found that prevalence of Candida infections is also more in this group. The study population age falls in early forties (42 years). Males are more commonly affected compared to females. In this study comparatively both males (39) and females (37) are equally affected.

Types of presenting lesions are pseudomembranous, erythematous and angular cheilitis. Current study documents pseudomembranous lesions as the common form of presentation. All the patients were on regular anti-retroviral treatment. Patients couldn't recall the antifungal prophylactic treatment in the course of the disease.

Among HIV positive individuals, *C. albicans* is known to be the predominantly identified species. However, there is increased reporting of non-albicans species in the last two decades as shown in Table 4. Menon T et al reported 74% of *C. albicans* and 22% of *C. tropicalis* in his study whereas Shobha Nadgir reported 66% of *C. albicans* and 33% of non-albicans in her study.

In our study *C. albicans* (77.66%) is the predominant isolate followed by non-albicans like *C. tropicalis* (21.05%) and *C. kefyr* (1.31%) as shown in table number (2).

Table:4 Various Studies showing predominant isolates on OPC from India.

Area	Year of study	Sample size	Predominant species
Chennai (Menon T et al.)	2001	46	<i>C. albicans</i> 74% <i>C. tropicalis</i> (22%)
Karnataka (Nadgir SD et al.)	2008	132	<i>C. albicans</i> (66.6%) Non-albicans (33.3%)

OPC: Oropharyngeal candidiasis.

As the immunity wanes recurrent oral thrush in HIV individuals is common. Current study, 46% (35 out of 76 isolated) of *Candida* species was isolated in the patients' group who had CD4 count <200 cells/mm³. And majority were *C. albicans* (25), followed by *C. tropicalis* (9) and *C. kefyr*. In our study *C. tropicalis* was found to be the more common non-albicans species causing OPC. The reason may be due to decreased immunity and prolonged anti-retroviral treatment replacing the *C. albicans* with non-albicans species (i.e. *C. tropicalis*).

In the patients with CD4 count between 200-500 cells/mm³, the most predominant species was *C. albicans* (7) and other species were *C. tropicalis* (4). In this study, *C. albicans* are most prevalent in this group. (delete this repetition) In the patients with CD4 count >500 cells/mm³ majority were *C. albicans*. And 6 patients were in the age group more than 45 years. This shows increased age predisposes to development of Candidiasis.

The study showed good sensitivity to voriconazole (82.4%) whereas fluconazole showed complete resistance (100%). Routinely antifungal susceptibility needs to be tested on all these resistant strains and their susceptibility pattern has to be noted. Most of the resistance is chromosomally mediated. The other important mechanism is the active efflux of these drugs which leads to reduced accumulation of drugs within the cell resulting in sub-lethal dose for the pathogen. Our study documents, decreased sensitivity to fluconazole in correlation with other study, this attributes to intermittent or prolonged prophylactic therapy given as treatment for suppression of infection.

Conclusions

This study documents the scenario in accordance to the changing trends of causative role of non-albicans in causing OPC which are resistant to azole drug fluconazole. Non-albicans *Candida* species are more common in patients with CD4 count <200 cells/mm³. ART significantly reduces the occurrence of OPC. As the CD4 count **declines**, rate of *Candida* infection rises and it is directly proportional with age. *C. albicans* isolate is attributed to endogenous infection.

Acknowledgement : Our heartfelt gratitude to all staff of KoIMS TH. Our sincere thanks to all patients who co operated in conducting this study. Our sincere thanks to ICMR for granting STS project No 2018 01455.

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