Distribution of *Candida* Species with Antifungal Susceptibility Isolated from Oral Thrush in People Living with HIV/AIDS and its Correlation with CD4 Count

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Abstract

**Background:** Oral Candidiasis is one of the most common opportunistic infection in People Living with HIV/AIDS (PLHA). Oral thrush is a reflection of the declining immunity in HIV positive patients. Distribution of the *Candida* species causing oral candidiasis along with their antifungal susceptibility will help in understanding the current scenario and aid in the treatment of the patients.

**Materials and Methods:** The aim of this study was to document the current prevalence of *Candida* species causing oral thrush in PLHA along with its susceptibility to antifungal drugs in a tertiary care hospital in South India. A total of 140 PLHA more than 18 years of age were screened for oral thrush clinically. Oral swabs were collected and processed by standard protocol in positive patients.

**Results:** Eighteen patients were found to be positive for oral thrush yielding 18 *Candida* isolates with 5 different species. *C.albicans* (66.6%) was the most common species followed by *C.tropicalis* (16.6%). Other species isolated include *C.dubliniensis*, *C. krusei* and *C. parapsilosis* 5.5% each. Of the 12 *Candida albicans*, 10 isolates were susceptible to all the antifungals tested while, one was resistant to ketoconazole and Fluconazole, and the other was resistant to Itraconazole. All the non-*albicans Candida* were susceptible to all the antifungals tested. CD4 count was less than 200cell/µL in 88.8% patients with oral Candidiasis with significant association.

**Conclusion:** The presence of oral candidiasis in a PLHA should alert the physician towards the advancing immunosuppression. Gradual emergence of non-*albicans Candida* species is a point concern.

**Keywords:** *Candida*; AIDS; Oral thrush

Introduction

AIDS (Acquired Immunodeficiency Syndrome) is essentially an infection affecting the immune system. The main clinical manifestation is due to progressive and profound defect in cell mediated immunity leading to infection by variety of normally innocuous agents which become the major source of morbidity and mortality [1]. HIV presently accounts for the highest number of deaths caused by any single infectious agent. The threat to their life being not from virus alone but due to opportunistic infections(OIs) and associated complications [2].

Oropharyngeal candidiasis is seen in about 3/4th of patients with HIV infection. In one-third of the patients it tends to recur and progress in severity with increasing immunodeficiency. Oral Candidiasis is frequently the first indication of immune impairment in HIV-infected patients [2,3]. It has been perceived that low CD4 counts and high plasma HIV RNA levels, both significantly correlate with oral candidiasis in HIV patients [4-6]. These observation suggest that decreasing the viral load and increasing the CD4 count by initiation of highly active antiretroviral therapy (HAART) would reduce the need for specific antifungal therapy. Hence early diagnosis of oral Candidiasis and initiation of HAART with restoration of immune status will help in improving the general well-being of the PLHA.

The most common candida species causing infection in AIDS patient is *C.albicans* though *C.glabrata*, *C.dubliniensis*, *C.parapsilosis* tend to cause infection in advanced disease [2,3].
The concern about the rising incidence of non-*albicans* *Candida* species is that they are generally less susceptible to the commonly used azole antifungal drugs [7,8].

With regards to the above, this study was conducted to determine the prevalence of oral Candidiasis, speciate the *Candida* isolates along with antifungal susceptibility testing and to study their correlation with the immune status (CD4 count) of PLHA in a tertiary care hospital, located in South India.

**Materials and Methods**

**Study Design and Population**

This cross-sectional study was conducted over a period of one year from July 2016 to June 2017 in the Institute of Microbiology in a tertiary care centre. Institutional Ethical Committee clearance was obtained. A total of 140 consecutive non-repetitive People living with HIV/AIDS attending Integrated Counselling and Testing Centre and those admitted in various wards aged more than 18 years were included in the study after obtaining informed written consent. Confidentiality and anonymity were maintained throughout the study. Personal and demographic data were obtained by structured questionnaire after obtaining informed written consent.

**Sample Collection and Processing**

Under aspetic precaution, 2ml blood collected in a EDTA vacutainer tube for CD4 count for all the PLHA irrespective of the presence of oral lesions. CD4 cell counts were measured by using Partec CyFlow counter (Partec CyFlow, New Delhi). The PARTEC CYFLOW Count System is an automated instrument designed for enumerating the absolute cell counts of CD4 in unlysed whole blood. It works with the principle of Flow Cytometry.

Oral cavity of the patients was examined for signs of candidiasis. Clinical Diagnosis of oral candidiasis was based on the characteristic appearance of the lesions along with the ease with which the whitish plaques can be scraped off.

The lesion was swabbed with two sterile cotton swabs, taking care not to contaminate the swab with saliva. One swab was used for Gram staining and observed for gram positive budding yeast cells with/without pseudohyphae and the other swab was inoculated onto Sabouraud’s Dextrose Agar, incubated at 37°C for 24-48 hours and observed for Cream coloured, smooth, pasty colonies.

**Speciation of Candida Isolates [7]:**

The Candida isolates were identified by Gram staining and other standard techniques like germ tube test, growth in CHROM agar Candida, chlamydospore formation and sugar fermentation test [7]. After the final identification, the isolates were stored at -80°C in 50% glycerol until susceptibility tests were performed.

**Antifungal Susceptibility Testing [9-11]:**

Antifungal susceptibility testing was done by disc diffusion method as per CLSI guidelines M44-A. Three to five colonies on SDA were emulsified in 2ml of sterile saline and matched to 0.5 McFarland turbidity standards. Lawn culture was made using a sterile cotton swab in three directions on Muller Hinton Agar plate supplemented with 2% glucose and 0.5µg/ml methylene blue, discs were placed on the surface of agar and incubated at 37°C for 24hours. The diameter of zone of inhibition was measured and compared with standard zones interpretive breakpoints published by CLSI M44-A2 guideline.

<table>
<thead>
<tr>
<th>Antifungal drugs</th>
<th>Disc content</th>
<th>Diameter of Zone of inhibition in mm</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Sensitive</td>
</tr>
<tr>
<td>Amphotericin B</td>
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</tr>
<tr>
<td>Nystatin</td>
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</tr>
<tr>
<td>Fluconazole</td>
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<tr>
<td>Ketoconazole</td>
<td>15 µg</td>
<td>^28</td>
</tr>
<tr>
<td>Clotrimazole</td>
<td>10µg</td>
<td>^20</td>
</tr>
<tr>
<td>Itraconazole</td>
<td>10µg</td>
<td>^23</td>
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</tbody>
</table>

**Table 1:** Antifungal Panel for *Candida* Species and their Interpretive Criteria.

DD-Dose Dependent.

**Statistical Analysis**

The statistical analysis was done using SPSS software. Age was presented as mean ± SD. The categorical variables such as gender and etiological agents were represented as percentages. Comparison of the mean age of the patients with and without oral candidiasis was done using unpaired t test. Comparison of the Oral candidiasis among the different CD4 categories were done using Chi square test for trends. All P values < 0.05 were considered as statistically significant.

**Results**

A total of 140 patients were included in this study with 50 (35.7%) females and 90 (64.3%) males. The mean age of the patients included in this study was 39.76 ± 8.45. The minimum and the maximum age of the patients were 22 and 70, respectively. Of the 140 patients, 18 (12.8%) patients presented with oral candidiasis.
Discussion

HIV infection is not only associated with high Candida colonization rate but also with development of overt disease like oral thrush [12]. Candidiasis being a frequent infection among PLHA, has been documented in up to 70% of the patients infected with HIV [2]. The incidence, prevalence and severity of Candida infection is inversely proportional to the CD4 count of the PLHA. The treatment of Candidiasis is hampered by delay in diagnosis and lack of species level identification especially in PLHA in whom non-albicans Candida species are in high prevalence causing serious infection and exhibiting relatively high resistance to commonly used azole antifungal drugs. With these view, this study was conducted to determine the profile and antifungal susceptibility of Candida species from oral thrush of People Living with HIV/AIDS (PLHA) along with correlation of their immunological status.

The mean age of PLHA in this study was 39.76 ± 8.45 with age range of 20-70 years and the age group of 20-45 years being the most commonly affected. Male preponderance was observed in this study with a male to female ratio of 1.8:1 which correlates with the demographic variables of PLHA in our country. It also highlights the increased risk in males in comparison with females attributing to their risky behaviours and jobs. Similar findings was found by Anwar et al., Nissapatorn et al. and Maheswari et al. who studied the opportunistic infections spectrum and candida profile in PLHA [12-14].

Of 18 Candida species isolated from oral lesions, 12 were Candida albicans (66.6%) while 6 were non albicans Candida species (33.3%) which were consistent with other studies done by Sangeetha et al., Nadeem et al., Francis et al. and Kaur et al. [15-17]. Among the 18 Candida isolated from Oral thrush of PLHA, Candida albicans was the most frequently isolated species accounting to 66.6%, followed by C.tropicalis (16.6%), C.dubliniensis (5.5%), C.krusei (5.5%) and C.parapsilosis (5.5%).

Isadora et al Mexico, demonstrated the same with C.albicans (71.8%) being the most common species followed by C.tropicalis (12.2%), C.glabrata (8.3%), C.parapsilosis (2.2%), C.krusei (1.7%) and C.guilliermondii (1.1%) [18]. And Faseela et al. Karnataka [19], the study done with 40 Candida isolates from oral thrush 60% were C.albicans and was the predominant species, followed by 15% C.tropicalis, 10% C.glabrata, 5% C.dubliniensis, 5% C.krusei, 2.5% C.lusitaniae and 2.5% C.parapsilosis which was similar to the findings in the present study. Though all these studies show Candida albicans as the most common species, an increasing trend in the prevalence of non-albicans Candida is being observed.

The Antifungal susceptibility test performed in this study revealed that among 12 C.albicans, 10 isolates were sensitive to all the antifungals tested while 2 were resistant of which, 1 isolate was resistant to Fluconazole and Ketaconazole and the other isolate was resistant to Itraconazole. All the non-albicans Candida were susceptible to all the antifungals tested.

Table 2: Profile of Fungal Pathogen Causing OIs (n=18).

Among the Candida species isolated, Candida albicans was the most common species responsible for Oral Candidiasis followed by Candida tropicalis.

Among the 12 Candida albicans tested, 10 isolates were susceptible to all the antifungals. Out of the two resistant isolates, one was resistant to ketoconazole and Fluconazole, and the other was resistant to Itraconazole. All the non-albicans Candida were susceptible to all the antifungals tested.

Table 3: Association of CD4 Count and Candidiasis (n=140).

There was significant association between CD4 count and the prevalence of candidiasis (P value 0.007). Of the 18 patients with candida infections, 16 patients had CD4 count < 200 cells/μl, while 2 patients had CD4 count between 200 – 499 cells/μl. Candida infection was not found in the 20 patients with CD4 count ≥ 500 cells/μl.
infections.

The mean CD4 cell count in PLHA with Candidiasis was 139cells/μL with a range varying from 35 to 328 cells/μL. There was significant association between decrease in CD4 count and prevalence of Candidiasis in the present study. This demonstrates that cell mediated immune response plays a significant role in preventing candidiasis. This observation was further supported with the studies done by Ajay kumar et al., Li Y-Y et al., Maurya et al. and Hung et al. who reported a strong correlation of prevalence of candidiasis in PLHA with low CD4 count [20-23].

There are few limitations in this study. The sample size was small, hence further studies with a larger sample size are required to substantiate the results. There are chances for inaccuracies and misdiagnosis of the Candida species due to lack in use of genotypic methods to identify the species.

Conclusion

This study gives an insight in the current prevalence profile of Candida species causing oral thrush in People living with HIV/AIDS from a tertiary care hospital in Chennai. Oral Candidiasis is associated with low immune status, hence its incidence should alert the physician of declining immune function and anti-retroviral resistance in patients on HAART. There is an increasing trend in non-\textit{albicans Candida} causing oral thrush which are less susceptible to the antifungal agents. The use of reliable and accurate diagnostic methods to identify the non-\textit{albicans Candida} could assist the physician in treating the patient. Practicing the use of antifungals in patients with severe, persistent, and invasive infections will avoid the incidence of antifungal resistance in the long run.

References