GAVIN PUBLISHERS

OPEN ACCESS

Current Research on HIV/AIDS

Verma SP. Curr Res HIV: CRHA-121.

DOI: 10.29011/2575-7105.100121

Research Article

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

Justine Auxilia Irene Chinnappan*, Lakshmipriya N and Umadevi U

Institute of Microbiology, Madras Medical College, Chennai, India

***Corresponding author:** Justine Auxilia Irene Chinnappan, Institute of Microbiology, Madras Medical College, Chennai, India

Citation: Chinnappan JAI, Lakshmipriya N, Umadevi U (2020) Distribution of *Candida* Species with Antifungal Susceptibility Isolated from Oral Thrush in People Living with HIV/AIDS and its Correlation with CD4 Count. Curr Res HIV: CRHA-122. DOI: 10.29011/2575-7105.100122

Received Date: 12 October, 2020; **Accepted Date:** 17 October, 2020; **Published Date:** 22 October, 2020

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 *Candia* 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.dublinensis*, *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 Flucanazole, 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-*albican 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 18years 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℃ 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 0.5McFarland 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.

Antifungal drugs	Disc content	Diameter of Zone of inhibition in mm		
		Sensitive	Intermediate	Resistant
Amphotericin B	100IU	³15	14-Oct	<10
Nystatin	50µg	³15	14-Oct	£10
Fluconazole	25µg	³19	15-18(DD)	£14
Ketoconazole	15 µg	³28	21-27	£20
Clotrimazole	10µg	³20	19-Dec	£11
Itraconazole	10µg	³23	14-22(DD)	<13

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.

Pathogen	No. of pathogen (n=18)	Percentage (%)
Candida albicans	12	66.6
Candida tropicalis	3	16.6
Candida dublinensis	1	5.5
Candida krusei	1	5.5
Candida parapsilosis	1	5.5

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 Flucanazole, and the other was resistant to Itraconazole. All the non-*albicans Candida* were susceptible to all the antifungals tested.

		Candidiasis		P value
		Yes	No	
CD4 Cell Count	<200	16 (20.8%)	61 (79.2%)	0.007
	200 – 499	2 (4.7%)	41 (95.3%)	
	≥ 500	0 (0%)	20 (100.0%)	
Total		18	122	

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.

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 susceptibilty 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* isolates were sensitive to all the antifungals tested. Study done by Faseela *et al.,* demonstrated that 29% of *C.albicans* were resistant to Fluconazole and 1 *C.tropicalis* showed resistance to Amphotericin B while others were sensitive [19]. The resistant strains are not high in the isolates which can be attributed to the fact that, the infection is frequently treated by increasing the immune status of the patients with HAART rather than using antifungals. Antifungals are used only in patients with persistent and invasive

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-*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-*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

1. Fields BN, Knipe DM, Howley PM, Griffin DE (2001) Fields virology. 6th edition,Philadelphia: Lippincott Williams & Wilkins.
2. National AIDS Control Organization, Ministry of Health and Family Welfare, Govt. of India (2008) Guidelines for prevention and management of common opportunistic infections/ Malignancies among HIV infected Adults and Adolescents, New Delhi 1-3.
3. Panel on Opportunistic Infections in HIV-Infected Adults and Adolescents. Guidelines for the prevention and treatment of opportunistic infections in HIV-infected adults and adolescents:recommendations from the Centers for Disease Control and Prevention, the National Institutes of Health, and the HIV Medicine Association of the Infectious Diseases Society of America.
4. Gottfredsson M, Cox GM, Indridason ÓS, de Almeida GMD, Heald AE, et al. (1999) Association of plasma levels of human immunodeficiency virus type 1 RNA and oropharyngeal Candida colonization. Journal of Infectious Diseases 180: 534-537.
5. Nicolatou-Galitis O, Velegraki A, Paikos S, Economopoulou P, Stefaniotis T, et al. (2004) Effect of PI-HAART on the prevalence of oral lesions in HIV-1 infected patients. A Greek study. Oral Diseases 10: 145-150.
6. Liu X, Liu H, Guo Z, Luan W (2006) Association of asymptomatic oral candidal carriage, oral candidiasis and CD4+ lymphocyte count in HIV-positive patients in China. Oral Diseases 12: 41-44.
7. Chander J, Textbook of Medical Microbiology, 4th edition, Jaypee publications.
8. Mane A, Panchvalli S, Bembalkar S, Risbud A (2010) Species distribution & antifungal susceptibility of oral & colonising or infecting HIV infected individuals. Indian Journal of Medical Research 131: 836-838.
9. Clinical Laboratory Standards Institute (CLSI) (2009) Method for antifungal disk diffusion Susceptibility testing of Yeasts; Approved Guideline- second edition. CLSI document M44-A2 (ISBN 1-56238-703-0). Clinical Laboratory Standard Institute, Wayne: Pennsylvania.
10. Dota KFD, Freitas AR, Consolaro MEL, Svidzinski TIE (2011) A challenge for clinical laboratories: Detection of antifungal resistance in *Candida* Species causing vulvovaginal candidiasis. Laboratory Medicine 42: 87-93.
11. (2011) Susceptibility testing of yeasts.
12. Anwar KP, Malik A, Subhan KH (2012) Profile of candidiasis in HIV infected patients. Iran J Microbiol 4: 204-209.
13. Gugnani HC, Becker K, Fegeler W, Basu S, Chattopadhya D, et al. (2003) Oropharyngeal carriage of Candida species in HIV-infected patients in India. Mycoses 46: 299-306.
14. Maheshwari M, Kaur R, Chadha S (2016) Candida Species Prevalence Profile in HIV Seropositive Patients from a Major Tertiary Care Hospital in New Delhi, India. J Pathog 2016: 6204804.
15. Patel SD, Kinariwala DM, Javadekar TB (2011) Clinico-microbiological study of opportunistic infection in HIV seropositive patients. Indian J Sex Transm Dis 32: 90-93.
16. Jeddy N, Ranganathan K, Joshua E, Devi U (2011) A Study of Antifungal drug susceptibility of Candida isolated from Human immunodeficiency virus infected patients in Chennai. South India. J Oral Maxillofac Pathol 15: 182-186.
17. Kwamin F, Nartey NO, Codjoe FS, Newman MJ (2013) Distribution of Candida species among HIV-positive patients with oropharyngeal candidiasis in Accra, Ghana . J Infect Dev Ctries 7:41-5.
18. Isadora Clark Ordonez , Olga A Calllejas-Negrete, Elva T Arechiga-Carvajal, Rosa R Mourino-Perez (2017) Candida species diversity and antifungal susceptibility patterns in oral samples of HIV/AIDS patients in Baja California, Mexico. Medical Mycology 55: 285-294.
19. shafi FT, Padmaraj SR, Mullessery NP (2017) Species distribution and antifungal susceptibility pattern of Candida causing oral candidiasis among hospitalized patients. Amhsjournal.org 247-251.
20. Sarvepalli AK, Dharana PK (2017) Spectrum of opportunistic infections with correlation to CD4 count in newly diagnosed HIV seropositive cases ijam 4: 1.
21. Li YY, Chen WY, Li X, et al. (2013) Asymptomatic oral yeast carriage and antifungal susceptibility profile of HIV-infected patients in Kunming, Yunnan Province of China. BMC Infectious Diseases 13: 46.
22. Maurya V, Srivastava A, Mishra J, Gaind R, Marak RSK, et al. (2013) Oropharyngeal candidiasis and Candida colonization in HIV positive patients in northern India. The Journal of Infection in Developing Countries 7: 608-613.
23. Hung CC, Yang YL, Lauderdale TL, McDonald LC, Hsiao C, et al. (2005) Colonization of human immunodeficiency virus-infected outpatients in Taiwan with Candida species. Journal of Clinical Microbiology 43: 1600-1603.