

Full Length Research Paper

Prevalence and distribution of Candida Species in HIV infected persons on antiretroviral therapy in Jos

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Abstract

Oropharyngeal candidiasis (OPC) is commonly associated with HIV. The causative agent is a yeast strain that is originally present as a commensal of the oral cavity. Most species of the genus *Candida* that cause OPC in HIV patients if not identified and properly treated with the drug of choice could result in resistance to the drugs and make treatment very difficult. This study was carried out to establish the species spectrum of the common yeasts associated with OPC in HIV patients on antiretroviral treatment in Jos. A total of 248 samples was collected; one hundred and ninety eight (198) Throat swabs from HIV seropositive males and females at the 2 hospitals. 50 control samples from HIV seronegative persons were collected. The samples were cultured on Sabourauds dextrose agar and *Candida* Species isolated were characterized using germ tube test and sugar fermentation tests. Out of 248 subjects (HIV positive and HIV negative individuals) were examined for oropharyngeal candidiasis, 24 (9.68%) yielded *Candida* growth. The age group 36-45 years accounted for the highest number 12 (50%) of isolates. *Candida albicans* accounted for 19 (79.2%) of the isolates, *Candida pseudotropicalis* 2 (8.3%), while *Candida tropicalis*, *Candida parapsilosis* and *Candida guilliermondi* had 1 (4.2%) isolates each. More women 18 (7.26%) had oropharyngeal candidiasis than men 6 (2.41%). 10% of the population studied were found to be colonized with *Candida*. HIV patients whether or not on drugs were predisposed to oropharyngeal candidiasis. *C. albicans* is the commonest species associated followed by, *C. pseudotropicalis*, *C. tropicalis*, *C. parapsilosis* and *C. guilliermondi*. Among patients on ART, *Candida albicans* was most prevalent 14 (58.33%). *Candida guilliermondi* was the only species of *Candida* found in HIV positive patients not on ARV. *Candida albicans* still remains the leading cause of oropharyngeal candidiasis in HIV infected persons within the study population. Constant identification of isolates of yeasts infecting HIV infected persons and the immune compromised will further enhance the appropriate treatment and minimise the speedy emergence of antifungal resistance.

Keywords: *Candida* species, characterization, oropharyngeal candidiasis.

INTRODUCTION

Oropharyngeal candidiasis (OPC) is the most frequent opportunistic fungal infection among Human Immunodeficiency Virus (HIV)-infected patients (Lattif et al., 2004). It has been estimated that more than 90% of HIV infected patients develop this often debilitating infection at some time during the progression of the disease (Samaranayake et al., 2000; Kamiru and Naidoo, 2002). The most common agents are *Candida albicans*,

C. glabrata, *C. krusei*, *C. tropicalis*, *C. parapsilosis*, *C. dubliniensis*, (Colman et al., 1998; Sant'Ana et al., 2002). *C. guilliermondi*. Non *albicans* species are implicated with greater frequency as opportunistic pathogens associated with diseases especially in immunocompromised hosts Baradkar and Kumar, 2008. OPC typically occurs when the patient's CD4 counts are 200 or less. It is considered a clinical marker of immune failure in HIV/AIDS patients but the incidence of oral infection in HIV infected patients has not been well documented (Repentigny et al., 2002). Oral candidiasis is frequently complicated with esophageal candidiasis which may limit food consumption and lead to weight loss, threatening the

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general health of the HIV infected persons (Dunic , et al 2004) The prolonged management of oral candidiasis might cause the development of drug resistant candidiasis and there have been reports of emergence of resistance to antifungal agents in HIV patients with OPC due to resistant *Candida* isolates and this has made treatment with standard doses for standard duration time very difficult. Prolonged use of antifungal agents may predispose to a shift in non-*albicans* species associated with refractory and recurrent infections (Hamza, *et.al.* 2008). Of concern is the full potential impact of antiretroviral therapy on the ability to reconstitute immunity and therefore reduce the incidence of oral candidiasis has been hampered by suboptimal adherence, toxicity and resistance to the antiretrovirals as well as limited availability of these treatments in developing countries where most HIV patients reside (Dabyshire, 2000). OPC increases morbidity, mortality and also reduces the quality of life of HIV- infected patients. The different species of *Candida* that cause OPC in HIV patients if not identified and properly treated +with the drug of choice could lead resistance to the drugs and make treatment very difficult. This study was carried out to establish the prevalent Species of *Candida* in HIV patients taking ART in Jos.

MATERIALS AND METHODS

Study Area

This study was carried out in Jos plateau in central Nigeria; it is about 1800m above sea level. It is situated between latitude 09⁰00-10⁰30N and longitude 08⁰30-09⁰80E. It has annual mean temperatures at 13.9⁰C and annual temperature are average between 21⁰-24⁰C especially at night. Occasionally, ground temperature drops to below freezing point. The study area experiences three seasons in the year; hot dry season, cold season and rainy season. The average annual rainfall is 750mm and the average annual relative humidity is 65%.

Study Population

One hundred and ninety eight, HIV seropositive males and females aged 18-70 years receiving antiretroviral therapy at Plateau State Specialist Hospital and Faith-Alive Foundation, Jos were recruited for this study. Fifty Control samples from HIV seronegative persons was collected.

Collection of Samples

Throat swabs were collected from the subjects using a spatula and sterile cotton swab. The spatula was used to

hold down the tongue to avoid picking normal flora from the tongue. The sterile evepon swab sticks were used to gently swab the oropharyngeal surface and removed in such a way that it doesn't touch the tongue and quickly inserted into the sterile swab stick cover to avoid drying before it was transported to the laboratory.

Isolation of *Candida* Species Associated with Oropharyngeal Candidiasis in HIV Patients.

The organisms were cultured by carefully streaking the swab stick on the petri dish containing Sabouraud Glucose Agar was used near a heat source to avoid contamination and kept in the incubator at 37⁰C for 3-5 days.

Subculturing

After culturing for 3-5 days, the colonies that are creamy, pasty and creamy were sub cultured on saborauds dextrose agar to get pure cultures which was used for analysis.

Characterization of *Candida* Species Associated with Oropharyngeal Candidiasis in HIV Patients

The isolated colonies were gram stained to identify yeast cells of the genus *Candida* which grew as oval budding yeast cells.

Germ Tube Test

Candida albicans from samples suspected to be *Candida* species by using Germ tube test briefly described; 0.2 ml of sterile human serum was transferred into a test tube. The organism was introduced into the test tube containing the serum using a sterile inoculating needle. It was incubated at 37⁰C for ninety minutes. A drop of the sample was placed on a clean slide and viewed under x 10 and x 40 objective of the microscope. The microscopic observation revealed short hyphal strands which were not constricted at the junction of the blastoconidia and the germ tube.

Biochemical Test for the Identification of *Candida* Species

Sugar Fermentation and Assimilation

Non-*albicans* species of *Candida* were characterized using sugar fermentation and assimilation tests. Sugar solution was prepared in peptone water. Two grams of peptone water and 1g of NaCl was dissolved in 200ml

Table 1. Age and Sex Distribution of HIV-1 Positive patients on ART at PSSH and Faith Alive Foundation in Jos

Age Group	Male	Females	Total No of samples
	No of samples	No of samples	
15-25	8	21	29
26-35	35	70	105
36-45	14	61	75
46-55	3	8	11
56-65	0	4	4
66-75	0	0	0
Total	42	206	248

Age group (years) - age of study subject

Sample numbers (MALES)-number of male subjects

Sample number (FEMALES) - number of female subjects

Total sample number- total number of male and female subjects

water and autoclaved at 121°C for 15 minutes and allowed to cool. 2.5g of each sugar was dissolved in 25ml distilled water and sterilized by filtration. To prepare 0.5% peptone water, sugars, 100ml peptone water was aseptically added to the sugar solution. Four ml of the peptone water sugar was aseptically dispensed into well labeled bijoux bottles containing an inverted Durham tube (the Durham tubes were sterilized in the bottles by autoclaving). The organisms were inoculated in the peptone water sugar from the subculture using a sterile inoculating needle. It was kept in the incubator at 37°C and observed for gas formation which indicates fermentation and turbidity signifying assimilation.

RESULTS

Prevalence of *Candida* Species

Out of the 248 subjects examined for oropharyngeal candidiasis, 24 (9.68%) yielded *Candida* species. Males accounted for the higher prevalence 6(14.29%) compared to 18(8.74%) in females. The age group 40-44 years accounted for the highest prevalence 6 (25.00%) followed by the age group 35-39 6(11.32%). Other age groups had the following prevalence 45-49 4(10.81), 25-29 3(12.50%), 30-34 3(4.05%) and ≥50 2(13.33%). The lower age brackets (14-19 years and 20-24 years) yielded no *Candida* growth (Table 1).

Prevalence of *Candida* Species in relation to HIV serostatus and Therapy

Of the 24 *Candida* species recovered from the 248 subjects examined for oropharyngeal candidiasis, 18(11.39%) isolates were obtained from the 158 HIV seropositive subjects on ART while 6(15.00%) were

obtained from the 40 HIV seropositive patients not receiving ART. No isolate was recovered from the HIV seronegative (control subjects) (Table 2).

Characteristic of *Candida* species

Of the 24 *Candida* species isolated, 19 (79.17%) were germ tube formers and were identified as *Candida albicans*. The non-albicans species identified were: *Candida tropicalis* 1(4.17%), *Candida pseudotropicalis* 2(8.33%), *Candida parapsilosis* 1(4.17%), and *Candida guilliermondii* 1(4.17%) (Table 3).

Distribution of *Candida* species in relation HIV serostatus and therapy

Candida albicans was the most prevalent species isolated from both HIV seropositive subjects on ART (77.78%) and those not on ART (83.33%). Other species recovered from patients on ART were; *Candida pseudotropicalis* 2(11.11%), *Candida tropicalis* 1(5.56%) and *Candida parapsilosis* 1(5.56%). *Candida guilliermondii* was the only non-albicans species found in patients not receiving ART (Table 4).

DISCUSSION

The carriage rate of *Candida* in the the mouth of HIV seropositive people in Jos is 9.68%, this rate is lower than the 34.4% reported by Enwuru et al., 2008 from HIV seropositive people in Lagos Nigeria, Agwu et al. (2011) in Uganda found *Candida* species in 52% of their subjects who had HIV and were on antifungal therapy, carrier rate was higher than in studies from Thailand (adults 66.6 % and children 70 % (Teaipaisan and

Table 2. Germ Tube Formation, Sugar Fermentation and Assimilation of *Candida* Species Isolated From HIV Positive Patients In Jos

S/N	Gro wth On SDA	Ger m tube test	Pse u- Hyp hya e	SUGAR ASSIMILATION							FERMENTATION					SPECIES
				Dex	Suc	Lac	Gal	Xyl	Rafi n	Ara b	Dex	Suc	La c	Gal	Xyl	
1	+	-	+	+	+	-	+	-	-	+	F	F	-	F	F	<i>Candida tropicalis</i>
2	+	-	+	+	+	+	+	+	+	-	F	F	F	F	F	<i>Candida pseudotropicalis</i>
3	+	-	+	+	+	+	+	-	+	-	F	F	F	F	F	<i>Candida pseudotropicalis</i>
4	+	-	+	+	+	-	-	-	-	+	F	-	-	-	-	<i>Candida parapsilosis</i>
5	+	-	+	+	+	-	+	+	+	-	F	F	-	F	-	<i>Candida guilliermondi</i>
6-24	+	+	+							√						<i>Candida albicans</i>

Key
 + implies Assimilation ; - implies No Assimilation; F implies Fermentation;
 Dex- dextrose, Suc-sucrose, Gal- galactose. Xyl- xylose, Raff-raffinose, Lac- lactose, Ara- arabinose
 √ Fermentation and Sugar assimilation not carried out

Table 3. Species of *Candida* Obtained From 248 HIV Infected Patients and HIV Negative Persons Attending Plateau State Specialist Hospital and Faith Alive Foundation

Species of <i>Candida</i>	Number of Isolates	% Number of Isolates
<i>Candida albicans</i>	19	79.2
<i>Candida pseudotropicalis</i>	2	8.3
<i>Candida parapsilosis</i>	1	4.2
<i>Candida tropicalis</i>	1	4.2
<i>Candida guilliermondi</i>	1	4.2

Species of *Candida*- organisms isolated
Number of isolates- frequency of occurrence of *Candida* species
% number of isolates- % frequency of occurrence of *Candida* species

Table 4. Species Distribution of *Candida* Species in HIV Positive and HIV Negative

Subject	Species	Number of Isolates	% Number of Isolates
Isolates On ARV	<i>Candida albicans</i>	14	58.8
	<i>Candida pseudotropicalis</i>	2	8.3
	<i>Candida tropicalis</i>	1	4.2
	<i>Candida parapsilosis</i>	1	4.2
Not on ARV	<i>Candida albicans</i>	5	20.8
	<i>Candida guilliermondi</i>	1	4.2

KEY
Subjects- HIV patients on ARV and those not on ARV involved in the study
Species- species of organism isolated
Number of isolates- frequency of occurrence of *Candida* species isolated
% number of isolates- % frequency of occurrence of *Candida* species isolated

Nittayananta, 1998; Pongsiriwet et al., 2004), Hong Kong (54.8 %) Tsang and Samaranayake, 2000, Italy (61.9 %) Campisi et al., 2002, Germany (73.8 %) Schmidt-Westhausen et al., 1991 and India (65.3 %) Gugnani et al., 2003. The low prevalence of oral candida in this study

may be attributed to patients strict adherence to their antiretroviral regimen. The carriage rate was found to be more in women(7.26%) than in men (2.41%), this is probably because most men rarely go for routine check ups until the disease has reached symptomatic stage and

as at the time of this study only a few men concented. People around the age range of 36-45 accounted for the highest prevalence rate. Among the patients examined only two reported having having pain when swallowing food and having altered taste, this suggest that oropharyngeal candidiasis in most cases is asymptomatic.

The results of this study provides evidence of the isolation of *Candida* species in the oral pharyngeal swabs of HIV infected persons on Antiretroviral therapy (ART) as well as in those not on ART in Jos. HIV seropositive people whether or not on ART are predisposed to oropharyngeal candidiasis. This does not agree with most studies, Cassone et al. (1999) reported that following the introduction of highly active antiretroviral therapy (HAART) there was a reduction in occurrence of opportunistic infections, prevalence of oral manifestations and oral candidiasis. Arribas et al., also suggested that that the reduction in the frequency of oral candidiasis was only related to immunological improvement after introduction of antiretroviral therapy including protease inhibitor (PI), which increases the number of CD4+ T cells. In another study out of 69.4% of patients undergoing HAART and 59.5% of patients without this regimen. However, candidiasis was less frequent in patients undergoing HAART (36.1%) than in patients without antiretroviral therapy (45.9%) (Sanchez et al., 2005). However some HIV positive patients with relatively high CD4+ cell counts develop oral candidiasis (Kahn and Walker, 1998). Control samples obtained from HIV sero-negative persons did not show any growth on Saboraud's dextrose agar (SDA). This differs from the reports of Hamza et.al, (2008) that *Candida* species can be found in both HIV positive and HIV negative persons with a significant difference in oral carriage of the organism.

Candida albicans was the most frequently isolated species. This also confirms findings of Enwuru et al. (2008) and Rejane et al. (2002) who reported 40.5% and 57.4% in Lagos and Brazil respectively. Similarly, Nweze and Ogbonnaya (2011) evaluated the species spectrum of yeast cells occurring in oral lesions of HIV positive persons in Nigeria and reported finding 60% yeast cells carriage in those on ART and *C.albicans* to be the most predominant species occurring. *C. albicans* is followed in frequency by, *C. pseudotropicalis*, *C. tropicalis*, *C. parapsilosis* and *C.guilliermondii*. Baradkar and komar, (2007) however, found *C. parapsilosis* to be second most most frequently isolated species after *C. albicans*. Costa et al. (27) also reported that *Candida albicans* presented the highest frequency (50%) whereas non-*C. albicans* species were represented by *C. tropicalis* (20.9%), *C. parapsilosis* (19.3%), *C. guilliermondii* (4.8%), *C. lusitaniae* (1.6%), *C. krusei* (1.6%), and *C. kefyr* (1.6%) were present. *Candida guilliermondii* was not present in HIV patients on ART but was isolated in patients not on ART, although *Candida albicans* still

remains the leading cause of oropharyngeal candidiasis in HIV infected persons. Care must be taken in identifying these species especially amongst persons that are immunocompromised, in order to guide treatment.

CONCLUSION

Several *Candida* species causing oropharyngeal candidiasis exists in about 10% of HIV/AIDS infected patients attending Plateau State Specialist Hospital and Faith- Alive Foundation, Jos. Routine checks for opportunistic infections including oropharyngeal candidiasis is important and should be carried out as at when due because it helps to monitor disease progression and it prevents complications such as candidemia. Identifying *Candida* to its species level is important because it helps guiding appropriate treatment. HIV patients not on drugs should also be screened for oropharyngeal candidiasis because the presence of OPC in such individuals could be an indication to start antiretroviral therapy.

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