

Prevalence of oral candida albicans infection in HIV sero-positive patients in Abakaliki

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Abstract: Oral thrush is commonly associated with HIV infection. The causative agent is a yeast strain that is originally a commensal of the oral cavity. Most species of the genus *Candida* that causes oral candidiasis in HIV patients if not properly identified and treated with the drug of choice could result in resistant to the drugs and make treatment very difficult. This study was carried out to establish the species spectrum of the common yeast (*Candida albicans*) associated with oral candidiasis in HIV patients on antiretroviral treatment in Abakaliki. A total of 240 samples were collected from HIV sero-positive males (64) and females (176) at the two hospitals. 40 control samples from HIV sero-negative persons were also collected. The samples were cultured on Sabouraud dextrose agar and *Candida* species were isolated and characterized using germ tube test and sugar fermentation tests. Out of the 240 subjects (HIV sero-positive patients) examined for oral candidiasis, the carriage rate of oral candidiasis were 12.5% (30/240). *Candida albicans* accounted for 80.00% in HIV sero-positive patients, followed by *Candida pseudotropicalis* (10.0%). More women, 21 (8.75) had oral candidiasis than men 9 (3.75%). HIV patients whether or not on drugs were predisposed to oral candidiasis. *C. albicans* (76.19%) is the commonest species associated with HIV infected patients on ART (Active Retroviral Therapy) followed by *Candida pseudotropicalis* (14.29%), *Candida tropicalis* (4.76%) and *Candida parapsilosis* (4.76%). Among the patients not on ART *Candida albicans* (88.89%) was most prevalent, followed by *Candida guilliermondii* (11.11%). *C. 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 minimize the spread emergence of antifungal resistance.

Keywords: Prevalence, Oral Candidiasis, HIV Sero-Positive Patients, ART, Abakaliki

1. Introduction

Oral candidiasis (also known as oral thrush) is a common opportunistic mycosis (yeast infection) of *Candida* species on the mucous membranes of the mouth (1-2). *C. albicans* is the most common species of yeast isolated from patients with oral candidiasis (3). The incidence of opportunistic infections due to *Candida albicans* and other *Candida* species has been increasing (4). Oral Candidiasis is the most common HIV related oral lesion and most patients are infected with a strain originally present as a commensal of the oral cavity (5).

The spectrum of *Candida* infection is diverse, starting from asymptomatic colonization to pathogenic forms. The low absolute CD4⁺ T-lymphocyte count has traditionally been cited as the greatest risk factor for the development of

oropharyngeal candidiasis and current guidelines suggest increased risk once CD4⁺ T lymphocyte counts fall below 200 cells/ μ (6).

The first step in the development of a candidal infection is colonization of the mucocutaneous surfaces (6). HIV infection is not only associated with increased colonization rates but also with the development of overt disease. During the course of HIV infection, the rate of *Candida* infection is inversely related to the CD4 counts of the patient which in turn depends on the use of Anti-retroviral treatment (7). HIV-positive patients carry more and a greater variety of yeasts than HIV-negative subjects. The prolonged management of oral candidiasis in HIV patients might cause the development of drug resistance candidiasis (8). Although the introduction of antiretroviral therapy (ART) has had a major impact on the infectious complications of AIDS (9), Candidiasis still remains a

common opportunistic infection in HIV-infected patients(6). Hence this present study is aimed to determine the prevalence of oral *Candida albicans* infection in HIV positive patients in Abakaliki.

2. Materials and Methods

2.1. Study Population

Two hundred and forty(240) HIV seropositive males and females within the age range of 19-70 years receiving antiretroviral therapy at Federal Teaching Hospital Abakaliki II(FETHA II) and General Hospital Ezzamgbo were recruited for this study, forty(40) control samples from HIV seronegative persons were also collected.

2.2. Sample Collection

Two hundred and forty(240) samples were obtained from the oral cavities of HIV-positive patients(men, 64; women, 176) with clinically important lesions of oral candidiasis receiving antiretroviral therapy at Federal Teaching Hospital Abakaliki II(FETHA II) and General Hospital Ezzamgbo(GHE). Forty(40) samples were also collected from HIV seronegative persons, which served as control. Informed consent was obtained from participants and procedures were performed according to FETHA II and GHE board of ethical committee. The oropharyngeal candidiasis(OPC) lesions in HIV-infected patients were diagnosed by an infectious diseases specialist on the basis of clinical presentation, findings of direct microscopic examination, and positive culture results.

2.3. Isolation of *Candida albicans* and Other Clinically Important *Candida* species

Specimens were obtained from the OPC lesions of the tongue or the buccal mucosa by using sterile cotton swabs. These swabs were incubated in Sabouraud's dextrose agar with chloramphenicol(Merck, Germany) at 32°C for 48 hours(under aerobic conditions) and in CHROMagar™ *Candida*(CHROMagar, France) at 35°C for 48 hours(in the dark) for production of species-specific colors.

Various chromogenic culture media capable of distinguishing *C. albicans* from other clinically important yeast strains are commercially available. Such media distinguish *Candida* strains from other yeast strains on the basis of the color changes produced by the *Candida* colonies, which are measured using pH indicators and by fermentation of specific compounds or chromogenic substrates for the presumptive identification of *C. albicans*, *C. pseudotropicalis*, *C. tropicalis*, *C. parapsilosis* and *C. guilliermondii*(10). We used a 10% KOH preparation and Giemsa stain for microscopic examination of pseudohyphae and yeast cell forms. We used sugar fermentation and sugar assimilation tests with RapID™ Yeast Plus System(Remel, USA) according to the manufacturer's instructions. Fresh yeast colonies were incubated with human serum at 37°C for 3 hours to test for germ tube formation. Development of filamentous-form cells and chlamyospore formation were evaluated by culturing the yeast isolates on Dalmau plates(cornmeal-Tween 80 agar) at 30°C for 48 hours(11).

Table 2. Gram tube formation, sugar fermentation and assimilation of *Candida* species isolated from HIV positive patients in Abakaliki

S/N	Growth on SDA	Germ tube test	Pseudohyphae	Sugar assimilation				Fermentation			
				Gly	Suc	Lac	Gal	Gly	Suc	Lac	Gal
1	+	-	+	+	+	-	+	+	+	-	+
2	+	-	+	+	-	+	+	+	+	+	+
3	+	+	+	+	-	+	-	+	+	+	+
4	+	+	+	+	+	+	-	+	-	-	-
5	+	-	+	+	+	-	+	+	+	+	-
6	+	-	+	+	-	+	-	+	-	+	-
7	+	-	+	+	+	-	+	+	-	-	+
8	+	+	+	+	+	-	+	+	+	-	+
9	+	+	+	√	√	√	√	√	√	√	√

Key: + = Positive, - = Negative

3. Results

Out of the two hundred and forty(240) samples of HIV positive patients on ART collected from FETHA II and GHE, 176 were from women and 64 were from men. The highest numbers of men screened were between the age ranges of 26-55(40), followed by 36-45(14), 15-25(6), and 45-55(2), while age range of 56-65(1) and 66-75(1) were the lowest screened. The highest number of female screened were within the age range of 26-35(125),

followed by 36-45(75), 15-25(26), 46-55(8), and 56-65(4), while 66-75(2) were the lowest screened.

The result of the Gram tube formation, fermentation and assimilation of *Candida* species isolated from HIV-seropositive patients is shown in Table 2 below

Out of the 30 *Candida* species isolated, 24(80.00%) were Gram tube formers and were identified as *Candida albicans*. The non *albicans* species identified were *Candida tropicalis* 1(3.33%), *Candida pseudotropicalis* 3(10.00%), *Candida parapsilosis* 1(3.33%), and *Candida guilliermondii* 1(3.33%) (Table 3).

Table 3. Species of *Candida* obtained from 240 HIV infected patients attending Federal Teaching Hospital Abakaliki II (FETHA II) and General Hospital Ezzamgbo (GHE)

Species of <i>Candida</i>	Number of Isolate	Percentage of Isolate (%)
<i>Candida albicans</i>	24	80.00
<i>Candida pseudotropicalis</i>	3	10.00
<i>Candida parapsilosis</i>	1	3.3
<i>Candida tropicalis</i>	1	3.3
<i>Candida guilliermondii</i>	1	3.3
Total	30	100

Candida albicans was the most prevalent species isolated from both HIV seropositive subjects on ART 17(76.19%) and that not on ART 8(88.89%). Other species recovered from patients on ART were, *Candida pseudotropicalis* 3(14.29%), *Candida tropicalis* 1(4.76%), and *Candida parapsilosis* 1(4.76%). *Candida guilliermondii* 1(11.11%) was the only non-*albicans* species found in patients not receiving ART (Table 4).

Table 4. *Candida* species distributed in HIV positive and HIV negative persons both on ART and not on ART

Subject	Species	Number of isolates	Percentage of isolates (%)
Isolates on ART	<i>Candida albicans</i>	16	76.19
	<i>Candida pseudotropicalis</i>	3	14.29
	<i>Candida tropicalis</i>	1	4.76
	<i>Candida parapsilosis</i>	1	4.76
Isolates not on ART	<i>Candida albicans</i>	8	88.89
	<i>Candida guilliermondii</i>	1	11.11

4. Discussion

The incidence of opportunistic infections due to *Candida albicans* and other *Candida spp.* has been increasing(4). *Candida albicans* is one of the most frequently isolated yeasts in clinical laboratories from HIV/AIDS patients(12). Studies have shown that this organism can account for up to 75% of the yeasts recovered from sites of infection(13). Rapid identification of candidiasis is important for the clinical management of immunocompromised patients(4). During the course of HIV infection, the rate of *Candida* infection is inversely related to the CD4 counts of the patient which in turn depends on the use of Anti-retroviral treatment(6).

Table 1. Age and sex distribution of HIV positive patients on ART at Federal Teaching Hospital Abakaliki II (FETHA II) and General Hospital Ezzamgbo (GHE)

Age Group (years)	Number of Male Screened	Number of Female Screened	Total
15-25	6	20	26
26-35	40	85	125
36-45	14	61	75
46-55	2	6	8
56-65	1	3	4
66-75	1	1	2
Total	64	176	240

The numbers of women screened in this study were found to be 176, while men 64 (Table 1). This is probably because most men rarely go for routine checkup, until the disease has reached symptomatic stage. Among the patients examined only three reported having pain when swallowing food and having altered taste, this suggested that oral thrush candidiasis in most cases asymptomatic.

The result of this study provides evidence of the isolation of *Candida* species in the oral pharyngeal candidiasis (OPC) of HIV infected persons on Antiretroviral Therapy (ART) as well as in those not on ART in Abakaliki. The current isolation rate of *C. albicans* in HIV seropositive patients was 80.00% which is close to the 82.2% reported from Iran(4), 71.25%, India(6), but higher than 61.8% reports from Ethiopia(5), 58.3%, Mexico(14) and Sao Paulo, Brazil(66.4%) (15). The non-*albicans* species identified were *Candida pseudotropicalis* (10.00%), *Candida tropicalis* (3.33%), *Candida parapsilosis* (3.33%), and *Candida guilliermondii* (3.33%) (Table 3). Non-*albicans* *Candida* as an agent of oral candidiasis in HIV/AIDS patients is documented (16, 14). Although *C. albicans* is the most frequently implicated pathogen in OPC, other *Candida spp.* are being increasingly associated with invasive candidiasis(17).

HIV seropositive people whether or not on ART are predisposed to oral candidiasis. This does not agree with most studies, (18) reported that following the introduction of highly active antiretroviral therapy (HAART) there was reduction in occurrence of opportunistic infections, prevalence of oral manifestation and oral candidiasis. Arribas *et al.* (19) also suggested 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 increase the number of CD4+ cells. However, some HIV positive patients with relatively high CD4+ cell counts develop oral candidiasis(6). Control samples obtained from HIV seronegative persons in this study showed growth on Sabaroud's dextrose agar (SDA), revealing *Candida albicans* (88.89%) and *Candida guilliermondii* (11.11%) (Table 4). This is in line with the report of Hamza *et al.* (8) that *Candida* species can be found in both HIV positive and HIV negative persons with a significant difference in oral carriage of the organism.

The carriage rate of oral candidiasis in HIV seropositive people in Abakaliki is 12.5% (30/240). This rate is higher than the 9.68% reported by Lar *et al.* (20) from HIV seropositive people in Jos Nigeria, but lower than the 34.4% reported by Enwuru *et al.*, (21) from seropositive people in Lagos Nigeria. Agwu *et al.* (22) in Uganda found *Candida* species in 52% of their subjects who had HIV and were on antifungal therapy. The carrier rate was also lower than in studies from Thailand, Adults 66.6% and children 70% (23-24). Hongkong(54.8%) (25), Italy(61.9%) (26), Germany(73.8%) (27) and India(65.3%) (28). The low prevalence of oral *Candida* in this study may be attributed to patient strict adherence to their antiretroviral regimen.

However, *Candida guilliermondii* was not present in HIV patients on ART on ART but was isolated in patients not on ART although *Candida albicans* still remains the leading cause of oral and vagina candidiasis in HIV infected person. Care must be taken in identifying species especially amongst persons that are immunocompromised, in order to guide treatment properly.

Various *Candida* species causing oral candidiasis exist in about 12.5% HIV/AIDS infected patients attending Federal Teaching Hospital Abakaliki II(FETHA II) and General Hospital Ezzamgbo(GHE). Routine checks for opportunistic infections including oral candidiasis are important and should be carried out at intervals to help monitor disease progression and also prevent subsequent complications such as candidemia. Identifying *Candida* to its species level is important because it helps guiding proper treatment.

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