Original Article

Distribution of Candida species among HIV-positive patients with oropharyngeal candidiasis in Accra, Ghana

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Abstract

Introduction: Oropharyngeal candidiasis is a common occurrence in the course of human immunodeficiency virus (HIV) disease progression. Changes in the clinical severity of oropharyngeal candidiasis and type of *Candida* species profile may be a reflection of immunological changes in patients. The aim of this study was to undertake a baseline *Candida* species identification for future reference.

Methodology: Oral swabs of 267 HIV-infected patients with oropharyngeal candidiasis were cultured and *Candida* species were identified by API 32 C.

Results: A total of 201 (75.3%) *Candida* species and 10 (3.7%) non candida fungi were identified. Twenty different *Candida* species were isolated. *Candida albicans* was the most prevalent species (68.5%) followed by *C. tropicalis* (7.4%), *C. krusei* (6.4%), *C. parapsilosis* (3.0%) and *C. sake* (2.5%). Other species ranged from 0.5% to 1.5%.

Positive culture was independent of whether patients were on anti-retroviral therapy or not.

Conclusion: Of all *Candida* isolates, 68.5% were identified as *C. albicans*. Since other uncommon species were also isolated, it may be necessary in this group of patients to identify *Candida* species causing severe infections.

Key words: OPC; HIV/AIDS; candidiasis; resource-limited countries

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Introduction

Candidiasis as a human infection may be caused by many different species within the genus. *C. albicans* remains the most predominant reported causative agent globally in more than half of established cases [1]. Oropharyngeal candidiasis (OPC) is the commonest fungal infection amongst HIV-positive patients worldwide. Infection can spread from the mouth through the pharynx to the oesophagus. A systemic infection of other parts of the body is not as common, but carries a high mortality of between 40% -100% [2].

The worldwide prevalence of OPC among HIV patients is 80% to 95% with *C. albicans* being the most common causative agent [3]. Globally, there is a gradual trend toward change in the *Candida* species with non-albicans *Candida* being associated with HIV/AIDS. Oral candidiasis has been reported as the third commonest clinical oral presentation in HIV-positive patients after melanosis and periodontal disease in Ghana [4]. Candidiasis is attributed to a reduction in host immune defenses. A change in the distribution profile of *Candida* species can be an indication of drug resistance or immunosuppression levels in a population. It could be a sensitive and specific indicator of a decrease in the number of CD4 cells and would show the onset of significant immune deficiency in people with HIV [5]. In some studies, a noticeable shift toward non-*albicans Candida* isolates has been associated with intrinsic or acquired antifungal resistance in several *Candida* species [6].

Therefore, taken together with other parameters of observed changes in the severity of OPC, CD4 cell count and viral load (if available), the change in species characteristics could give an indication of immunological changes occurring in the patient.

Ghana is a beneficiary of the global accessibility programme to antiretrovirals and it was started at this centre in December of 2003. The Accra centre caters to patients diagnosed with HIV/AIDS. The purpose of this study was to isolation and identify oral *Candida* to collect baseline data for future monitoring and evaluation of clinical conditions of patients.

Methodology

This was a single institutional study conducted over a six-month period from October 2008 to March 2009 at the Fever's Unit of the Korle-Bu Teaching Hospital (KBTH), Accra, Ghana. KBTH is a dedicated centre for the management of HIV/AIDS patients and runs both out-patient and in-patient services. The sample population was adults with HIV/AIDS attending the out-patient clinic. Inclusion criteria were a previous positive diagnosis of HIV, a presumptive diagnosis of OPC following an appropriate complaint made at the clinic visit, and no history of antifungal therapy within the two weeks prior to the attendance. Institutional ethical protocol was adhered to in addition to obtaining patient permission and consent.

The oral cavity mucosa from each participant was swabbed with sterile cotton, which was then aseptically cut into 10 ml brain heart infusion broth and incubated at 35-37° C for 18 to 24 hours. The broth was then sub-cultured onto Sabouraud dextrose agar (Oxoid, Basingstoke, UK) and incubated for 48 hours. Plates with no growth after 48 hours were re-incubated for a further one week.

Pure cultures of yeasts were identified using API ID 32C test kit strips (BioMerieux, Marcyl'Etoile, France). Colonies of yeast were emulsified in API suspension medium to form a turbidity equivalent to 2 McFarland Standard. Cupules of the test strips were filled with 135 ul of the test organism and incubated at 35-37°C for 24 to 48 hours. Test strips after incubation were read using Epiweb identification software (BioMerieux, Marcy l'Etoile, France) by manually entering the 10 digit numbers into the computer to reveal the identity of the organism to the species level.

Data was captured into MS Access (Microsoft, Redmond,USA) and analyzed using SPSS version 16 (IBM, Chicago, USA). Categorical variables were summarized by proportions and percentages. Comparison of proportions between groups was done by chi-square test, and the significant level set at $p < 0.05$.

Results

From October 2008 to March 2009, a total of 267 subjects were enrolled into the study. This population was urban and derived from the lower to the middle income group. The higher income and more educated group prefer to use private health care which they can afford. The catchment group therefore mainly included self-employed petty traders and low-level civil servants. Other centres exist throughout the country and those in rural settings serve mainly farming communities. There were 98 (36.7%) males and 169 (63.3%) females, giving a male:female ratio of 1 to 1.7 with an age range of 15 to 74 years. The female sample population peaked at the 30 to 39 years age group and the males at 40 to 49. The observed sex and age distribution pattern was similar to the reported national distribution pattern in Ghana [7] but differed from those from industrialized countries where HIV infection prevalence is reported more in males. Eighty-one (30.3%) of the participants were on highly active antiretroviral therapy (HAART) and 186 (69.7%) were not. Those from whom *Candida* was isolated were 201 (75.3%). Ten patients (3.7%) had fungal isolates which were not *Candida* species, and no fungal growth was found in 56 (21.0%) of the samples. No significant difference was found in fungal infection among those on HAART and those not on HAART ($\chi^2 = 0.21$, df = 1, $p = 0.647$).

Of the 186 patients who were not on HAART, 147 (79%) had fungal growth, while 66 (81.5%) of those on HAART had positive growth (Table 1).

Twenty different *Candida* species were identified. The most prevalent yeasts were *C. albicans* 139 (68.5%), followed by *C. tropicalis* 15 (7.4%), *C. krusei* 13 (6.4%), *C. parapsilosis* 6 (3%), and *C. sake* 5 (2.5%). Two subjects had two different isolates each, identified as *C. albicans/C. tropicalis* and *C. krusei/C. kefir* respectively, as shown in Table 2.

Other fungi identified were four isolates of *Cryptococcus laurentii,* two *Trichosporon asahii,* two *Zygosaccharomyces species,* and one isolate each of *Saccharomyces cerevisiae* and *Sporobolomyces salmonicolor*.

Table 1. Oral fungal infections among HIV patients

HAART Status	Isolate Yield		
	Positive n (%)	Negative n (%)	Total n (%)
+	66 (81.5)	15 (18.5)	81 (100)
-	147 (79)	39 (21)	186 (100)

Table 2. Distribution of *Candida* species among HIV-positive subjects

Organism	Number of isolates (%)
Candida albicans	139 (68.47)
Candida tropicalis	15 (7.39)
Candida krusei	13 (6.40)
Candida parapsilosis	6 (2.96)
Candida sake	5 (2.46)
Candida dubliniensis	3 (1.48)
Candida globosa	3 (1.48)
Candida famata	2 (0.99)
Candida glabrata	2 (0.99)
Candida guilliermondii	2 (0.99)
Candida lusitaniae	2 (0.99)
Candida norvegica	2 (0.99)
Candida dattila	1 (0.49)
Candida incons	1 (0.49)
Candida hellenica	1 (0.49)
Candida holmii	1 (0.49)
Candida kefyr	1 (0.49)
Candida pulcherrima	1 (0.49)
Candida valida	1 (0.49)
Total	203 (100)

Discussion

The commonest *Candida* species isolated was *C. albicans* (68.5%) followed by *C. tropicalis* (7.4%) and *C. krusei* (6.4%). In a previous study using the same management protocols but different clinical set-up conditions at the centre [8], 104 HIV/AIDS patients with no symptoms of oral candidiasis were matched against 101 non-HIV healthy individuals and were examined for the presence of clinical oral candidiasis and all had fungal swabs taken to assess carrier status. A prevalence of 87.5% *Candida* isolation was reported with *C. albicans* being the most prevalent at 57.1% in the HIV-positive group with 42.6% and 53.5% respectively in the control non-HIV healthy group in that study. That study also showed a difference in *Candida* species distribution between HIV-positive and non-HIV healthy controls despite both having *C. albicans* as the most prevalent species. *C. tropicalis* was reported in that series only in the HIV/AIDS subjects [8]. Similarly, this current study also isolated *C. tropicalis*.

C. albicans has been isolated as the most prevalent yeast from oropharyngeal swabs from several countries including Tanzania, South Africa, and Nigeria [9-11]. These are all resource-poor countries similar to Ghana. Positive culture was found to be independent of HAART status; however, in a previous study of ours, it was found to be related to CD4 cell count levels [4].

The Tanzanian study [9], which looked at 292 HIV-positive patients with primary and recurrent OPC, reported a *C. albicans* isolation rate of 84.5% compared to 68.5% in our study. It also reported *C. glabrata* as the second commonest isolate followed by *C. krusei*. Our study reported *C. tropicalis* as the second most common, with *C. glabrata* significantly lower. *C. dubliniensis* was similarly reported in both studies with very low isolation rates. The Tanzanian study also reported no significant difference in species distribution between the primary and recurrent OPC clinical groups, but the results showed a significant correlation of reduced susceptibility to azole antifungal agents in the recurrent OPC population. The South African study [10] examined 332 HIV-positive patients and 100 HIV-negative subjects for yeast carriage rate and, in the former group, also looked at the effect of antifungal exposure on carriage rate. The *Candida* carriage rate among the HIV-positive group with clinical manifestations of oral candidiasis was 81.3%, compared to 75.3% in our study. The South African study concluded that HIV-positive patients carry more and a greater variety of yeasts than HIV-negative subjects and exposure to antifungal drugs has no effect on the level of yeast carriage in HIV-positive patients. These patients did not have access to HAART.

An overview of the literature regarding oral fungal infections in HIV infected individuals in Africa [3] reveals, among others, articles on oral candidiasis prevalence and the predictive value of oral candidiasis for a diagnosis of underlying HIV as a marker of disease progression, the types of species isolated, and resistance of the yeasts to antifungal treatment. The prevalence of oral candidiasis in HIV-infected subjects across Africa was reported at between 1.5% and 94% from different studies. Comparisons are, however, complicated by differences in the selection of study groups and the identification methodologies employed. Conclusions included the fact that the literature has been sporadically documented. In addition malnutrition, which is widespread in the region, must be regarded as a confounding variable when reporting

on candidiasis in HIV infection subjects from resource-poor countries.

In a study using isolates from 40 different countries [16], 91.1% of the Candida isolates tested were susceptible to fluconazole,but other species such as *C. glabrata*, *C. krusei*, and other less common isolates exhibited a decreased susceptibility of 75% or less. A study from Ethiopia [17] also reported an 11.9% resistance rate of *C. albicans* to fluconazole. A study of clinical isolates of *Candida* from several different human sources in KBTH (2008-9) found that the majority of isolates were *C. albicans*, and resistance to fluconazole was 62%, while resistance to amphotericin B was 10%, and resistance to flucytosine was 30%. Twenty per cent of these strains of *C. albicans* were resistant to all three drugs tested (personal communication by Newman *et al*., Department of Microbiology, University of Ghana Medical School). A controlled group selection was not done in this study. In our study, only three (1.5%) of the isolates were *C. dubliniensis* and in the South African study [10], it was 0.3%. A study from Ireland reported a prevalence of 18% and 32% respectively of *C. dubliniensis* among HIV/AIDS positive patients asymptomatic and symptomatic with OPC [12], while in Argentina 13% of such isolates were obtained in HIV positive cases [13]. It is very likely that we might have detected some isolates with reduced susceptibility to antifungal agents if we had done susceptibility tests.

C. dubliniensis appears to be reported in association with HIV/AIDS infection mostly from the resource-endowed regions [14]. *C. dubliniensis* is described as having a close similarity phenotypically by colonial morphology to *C. albicans*, but it is genotypically different, making identification difficult except by molecular methods. Studies have reported that API 32C can correctly identify *C. dubliniensis* [15]. Therefore, if more were present, we would have detected these isolates of *C. dubliniensis*. The almost non-reportage of its incidence in other similar resource-limited geographic areas may be a result of lack of expertise or appropriate equipment, or a true reflection of *Candida* expressivity under different antimicrobial/antifungal exposures and HIV management protocols.

Although the API ID 32C test used in this study is reliable, it may be too expensive for routine testing in most resource-poor countries.

Conclusion

Studies evaluating changes in the distribution of *Candida* species in relation to the progression of HIV disease and development of resistance to HAART and/or antimycotics are rare in Africa. The advantage now in some African centres, such as the one in Accra, is that a reliable source of HAART is presently available. The challenge is to develop positive clinical predictive parameters to monitor the long-term effectiveness of HAART and possibly establish guideline protocols to help maintain its long-term effectiveness. This is in lieu of the unavailability of reliable but expensive microbiology laboratory investigative processes. A shift in *Candida* species distribution profile would be an initial trigger to search for cause. Presently across Africa, confounding factors of disease progression, other than probable changes in effectiveness of HAART, include immunological deterioration due to HIV infection itself, selective antifungal resistance development by *Candida* species, predominant type of diet, and malnutrition.

Outcomes may be difficult to generalize across neighbouring countries in Africa and even intra-country. An urban or rural setting could make a significant difference in anti-mycotic resistance development because of availability and exposure differences. Inter-country legislation governing the use and monitoring of anti-microbials varies widely.

Despite the challenges, developing guidelines for monitoring HIV disease progression in the course of HAART use and management at a more affordable cost would assure longer and more effective usage. Future follow-ups to assess significant shifts in *Candida* species distribution to act as a trigger for evaluation of cause in HIV management are proposed.

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