

Characterization of Candida Species from Oral Thrush in Human Immunodeficiency Virus (HIV) Seropositive and Seronegative Patients

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Abstract

Oral thrush caused by *Candida* species is usually an opportunistic infection. It is usually caused by *Candida albicans*, however, over the last decade reports of “non-*albicans* *Candida*” causing this condition are increasing. The present study was undertaken to assess the role of *C. albicans* vis á vis “non-*albicans* *Candida*” in the causation of oral thrush. Eighty patients with oral thrush were included in this study. Fifty patients were Human Immunodeficiency Virus (HIV) seropositive and 30 patients were HIV seronegative. Oral swabs collected from the lesions in both groups were subjected to a battery of standard mycological tests that yielded *Candida* species in 78.75% patients.

Isolation of *C. albicans* was 66% from the HIV seropositive group and 60% from the HIV seronegative group. The “non-*albicans* *Candida*” formed 24% of the *Candida* isolates from the HIV seropositive group while no “non-*albicans* *Candida*” was isolated from the HIV seronegative group. The commonest “non-*albicans* *Candida*” isolated were *Candida dubliniensis* (17.77%), *Candida parapsilosis* (6.66%) and *Candida tropicalis* (2.22%). It is important to specify the *Candida* as the “non-*albicans* *Candida*” group is more likely to develop drug resistance.

Introduction

Oral thrush is a common clinical manifestation of Candidiasis. It is usually an opportunistic infection associated with immunocompromised states such as diabetes, extensive antibiotic usage, malignancies and Human Immunodeficiency Virus (HIV) infection. In the last decade however, the incidence of infections due to *Candida albicans* have decreased and been replaced by “non-*albicans* *Candida*” species like *Candida tropicalis*, *Candida parapsilosis*, *Candida glabrata* and *Candida krusei*. The recent emergence of *Candida dubliniensis* as an opportunistic pathogen appears to coincide

with this apparent epidemiological shift. This newly recognized opportunistic pathogen has been linked to oral candidiasis in HIV- infected and Acquired Immunodeficiency Syndrome (AIDS) patients.

Among the many opportunistic infections observed in HIV seropositive patients, oropharyngeal candidiasis is the most common. It is observed in upto 90% of patients during the course of that disease. Oral candidiasis can progress to oesophagitis, this interferes with adequate oral intake which in turn contributes to the general morbidity in these patients. Although *C. albicans* remains the most frequent cause of oral candidiasis in AIDS patients, a number of reports have documented infections caused by “non-*albicans* *Candida*” species such as *C. tropicalis*, *C. glabrata*, *C. parapsilosis*, *C.*

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krusei, *C. lusitaniae* and newer species like *C. dubliniensis*. This newly recognized opportunistic pathogen has been linked to oral candidiasis in HIV seropositive patients. Increasing reports of “non-albicans Candida” species causing oral disease and the increasing number of HIV seropositive patients stimulated us to undertake a study to identify the different *Candida* species responsible for oral thrush in HIV seropositive and seronegative patients in our hospital.

Material and Methods

Two oral swabs each were collected from 80 patients clinically presenting with oral thrush from inpatient and outpatient departments of Sassoon General Hospitals, Pune. Fifty were from HIV seropositive patients and 30 were from HIV seronegative patients. (HIV status of the patient was confirmed by performing two tests i.e.: 1) ELISA (Enzaid HIV 1+2) manufactured by Span Diagnostics Ltd. and 2) Rapid test: HIV TRI- DOT manufactured by J.Mitra & Co. Ltd). The swabs were collected with all aseptic precautions from each patient and transported to the laboratory in sterile culture tubes. One swab was used to prepare a smear which was gram stained.¹ The second swab was plated on two slopes of Emmon’s modified Sabouraud glucose agar (SGA) supplemented with antibiotics (50 µg/ml of Chloramphenicol and 5 µg/ml of Gentamicin) and two slopes of SGA without antibiotics. Two sets of tube each incubated at 37° C and room temperature respectively and examined every 48 hours till growth was obtained. Growth of yeast-like organisms was confirmed by gram staining. These organisms were further speciated based on the following tests.

i) Germ tube test²:- A positive Germ tube test implied either *C. albicans* or *C. dubliniensis*. This was later confirmed by

other tests.

- ii) Growth on Cornmeal tween-80 agar test³:- This growth was examined for the following; Pseudohyphae or True hyphae, Blastoconidia, Arthroconidia, Chlamydospore and their arrangement amongst other features.
- iii) Carbohydrate assimilation test (Modified Wickerham Method)⁴:- The carbohydrates used were Glucose, Maltose, Lactose, Sucrose, Galactose, Xylose, Trehalose, Cellibiose.
- iv) Carbohydrate fermentation test⁴:- This test was performed as per the technique described by Larone DH.
- v) Urease test⁴:- This test was used to detect presence of urease enzyme produced by different *Candida* species. Christensens urea agar slants were used. Conversion of the yellow slope to pink or red was considered positive. A negative test was reported when there was no colour change observed.
- vi) Growth at 45°C on Potato dextrose agar (PDA)⁵:-This test was specifically performed to differentiate *C. albicans* from *C. dubliniensis*. The former grows at 45° C whereas the latter does not.
- vii) Morphology on Staib agar⁶:- This was another test which helped used to differentiate between *C. albicans* and *C. dubliniensis* on the basis of colony characteristics. Grey-white shiny colonies with smooth entire edges indicated *C. albicans* whereas colonies of *C. dubliniensis* were grey-white and rough with a fringe or hyphal halo observed under scanning microscope. After 96 to 120 hrs of incubation all isolates were further examined microscopically for pseudo and true hyphae, blastospores and chlamydospores. Table 1 summarizes the

Table 1 : Differentiation of Candida albicans and Candida dubliniensis in our study

Organism	Germ tube test	Microscopic Morphology on Cornmeal tween-80 agar at room temperature	Carbohydrate assimilation								Growth at 45° C	Staib agar morphology
			Dextrose	Xylose	Trehalose	Maltose	Sucrose	Lactose	Galactose	Cellibiose		
Candida albicans (51)	+	Pseudohyphae with terminal, intercalary or sessile chlamydo spores; clusters of lastospores at septa	+	+	+	+	+	-	+	-	Good growth (43) Poor growth (8)	Greyish-white mucoid colonies with fine edges. No chlamydo spore production.
Candida dubliniensis (8)	+	Pseudohyphae with abundant chlamydo spores in doublets, triplets and in small bunches. Blastospores present at the septa	+	-	+	+	+	-	+	*	No growth (7) Poor growth (1)	Greyish-white rough colonies with fine fringe. Chlamydo spore present.

+ → positive - → negative +* → all except one isolate did not assimilate Galactose.

tests performed to differentiate *C. albicans* from *C. dubliniensis* as the two resemble each other phenotypically.

Results

In the present study 76% patients were males and in the age group of 21-30 years. The 30 HIV seronegative individuals were immunocompromised due to other underlying conditions such as haematological malignancy 12.5% followed by Tuberculosis 7.5% and diabetes mellitus 6.25%. Of the 80 patients of oral thrush included in the study 63(78%) showed the presence of Candida species (45 from HIV seropositive group and 18 from the seronegative groups). In the HIV seropositive group majority of the isolates were *C. albicans* 33/45(74.3%) and the remaining were “Non-albicans Candida” i.e.

C. dubliniensis 8/45(17.8%) followed by *C. parapsilosis* 3/45(6.7%) and *C. tropicalis* 1/45(2.2%). All isolates obtained from the HIV seronegative group were *C. albicans* (Table 2). Table 1 shows the tests used to differentiate *C. albicans* from *C. dubliniensis* as *C. dubliniensis* closely resembles *C.*

Table 2 : Shows the different Candida species isolated in HIV seropositive and HIV seronegative patients

Organism	HIV Seropositive (50)	HIV Seronegative (30)
Candida albicans	33 (66%)	18 (60%)
Candida dubliniensis	8 (16%)	Nil
Candida parapsilosis	3 (6%)	Nil
Candida tropicalis	1 (2%)	Nil
No isolates	5 (10%)	12 (40%)

albicans phenotypically.

Discussion

Oral thrush is a common manifestation of immunosuppressed individuals. Infact, oro-oesophageal candidiasis is known to be an AIDS indicator disease. In the present study, the isolation rate of *Candida* species in patients with oral thrush was 78.75%. In the HIV seropositive group it was 90% and from HIV seronegative group it was 60%.

In the present study, *C. albicans* was the predominant (73.3%) isolate in both the groups and was the only *Candida* isolate in the HIV seronegative patients. Amongst the HIV positive patients it was present in 63.8% of patients. Similar observations in this group of patients have been made by Lopez-Dupla *et al* in 1992⁷ who reported 44.4% isolation of *C. albicans* from HIV seropositive patients. Korting *et al* in 1988⁸ reported 74% isolation of *C. albicans* from HIV seropositive patients. McCreary *et al* in 1995⁹ reported 100% isolation of *C. albicans* from 20 HIV seropositive patients. Their results were similar to the present study.

Though our study showed that all the isolates from the HIV seronegative group were *C. albicans*, varying results have been reported by other workers. Pathak *et al*¹⁰ reported a 43.47% isolation of *C. albicans* from HIV seronegative group. Gupta *et al*¹¹ reported 50% isolation of *C. albicans* from HIV seronegative group. Samonis *et al*¹² reported 86% isolation of *C. albicans* from HIV seronegative group.

“Non-*albicans Candida*” as the causative agent of oral thrush, have been reported from various sites ranges from 6.55%-70%. In our study the isolation rate of the “non-*albicans Candida*” species from HIV seropositive group were 12/50 i.e.24% while in HIV seronegative group it was nil.

Walmsley *et al*¹³ reported 20.6% isolation of “non-*albicans Candida*” from 97 HIV seropositive patients studied by them. McCreary *et al*⁹ reported 75% isolation rate of “non-*albicans Candida*” in their HIV seropositive patients. They also found that 70% of the patients were having more than one *Candida* species. Lopez –Dupla *et al*⁸ reported 6.5% of “non-*albicans Candida*”. However, majority of their patients had oesophageal candidiasis and they were all HIV seropositive.

In the present study, the major “non-*albicans Candida*” species isolated was *C. dubliniensis* 8/45 i.e. 17.77%. Walmsley *et al*¹³ isolated 7.2% of *C. dubliniensis* from HIV seropositive patients. Giamanco *et al*¹⁴ reported *C. dubliniensis* on six occasions from one asymptomatic HIV seropositive individual. Sullivan *et al*¹⁵ reported 64 *C. dubliniensis* from 55 Irish and Australian HIV seropositive patients. Same authors¹⁶ reported 11 isolates of *C. dubliniensis* from seropositive patients from Switzerland, UK and Argentina. Jabra-rizk *et al*¹⁷ reported 5 *C. dubliniensis* from HIV seropositive patients.

Though we did not encounter any *C. dubliniensis* isolates in our HIV seronegative group, there are some reports of this isolate from this group also. Polacheck *et al*¹⁸ reported 5 *C. dubliniensis* from different HIV seronegative hospitalized patients. Sullivan *et al*¹⁵ reported 2 *C. dubliniensis* from HIV seronegative Irish subjects. Most of the patients in these reports had an associated underlying disease, but one common factor was that all were treated with broad-spectrum antibiotics, which may have been a contributory factor in the outgrowth of *C. dubliniensis*.

The second most common “non-*albicans Candida*” species in our study was *C.*

parapsilosis 3/45 i.e.6.66%. Walmsley *et al*¹³ reported 3/97 i.e. 3.1 % of *C. parapsilosis* from HIV seropositive group. Lopez-Dupla *et al*⁸ reported 1/61 i.e.1.6% of *C. parapsilosis* from HIV seropositive group. Other “non-albicans *Candida*” species was *C. tropicalis* 1/50 i.e.2.22% in our study. Walmsley *et al*¹³ obtained 2/97 i.e. 2% *C. tropicalis* from HIV seropositive patients. Fichtenbaum *et al*¹⁹ isolated 5/154 i.e. 3.1% *C. tropicalis* from HIV seropositive patients. McCreary *et al*⁹ reported 3/20 i.e.15% of *C.tropicalis* from HIV seropositive patients.

“Non-albicans *Candida*” species which have been reported occasionally from oral thrush but were not encountered in our study were *C. glabrata*, *C. krusei* and *C. lusitaniae*.^{8,9,13,19}

The increasing emergence of “non-albicans *Candida*” thus seems to be associated with global HIV pandemic. The emergence of *C. dubliniensis* as a pathogen in HIV seropositive patients is a major cause of concern because of its ability to develop resistance to fluconazole. Since *C. dubliniensis* closely resembles *C. albicans* phenotypically it is possible that it is being missed in most laboratories which rely solely on germ tube test for the identification of *C. albicans*. Therefore, all germ tube test positive isolates should be screened on Staib agar and the identity of *C. dubliniensis* in suspect cases be confirmed by chlamydospore formation on cornmeal tween 80 agar, growth at 45° C on potato dextrose agar and carbohydrate assimilation tests at least.

The morbidity and risk associated with oral thrush, along with an increase in refractory oral Candidiasis as well as the high incidence of AIDS, makes it important that species identification of *Candida* isolates should be carried out in most microbiology laboratories.

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NEUROLOGICAL COMPLICATIONS IN CHIKUNGUNYA FEVER

Apart from typical clinical triad of high grade fever, arthralgia and rash of chikungunya infection we have observed a spectrum of neurological abnormalities in terms of altered mental functions, seizures, focal neurological deficit with abnormal CT scan of head and altered CSF biochemistry. Permanent neurological sequelae and even death has occurred. Typical clinical history of chikungunya infection, neurological complications with associated CSF abnormalities, supportive laboratory evidences, positive chikungunya IgM card test, exclusion of other causes and known predilection of arboviruses for CNS infection allows us to conclude the diagnosis of study cases as Chikungunya Encephalitis.

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