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Characterization of Candida Species isolated from samples taken from patients with known Immunocompromised state presenting with Oral Thrush

Authors
Prasad Mukul[1], Umarekar Poorva[2], Rangnekar Aseem[3]*
*Corresponding Author
Rangnekar Aseem

Abstract
Introduction: *Oral thrush caused by Candida species is mostly an opportunistic infection. Candida albicans is known to be the major cause of the infection, however, over the last few decades several authors have reported that "non-albicans Candida" are also increasing causing thrush.*
Aim: *To characterize the various candida species isolated in patients coming to dental OPD with known history of Immunodeficiency.*
Material and Methods: *A retrospective hospital based study, by collecting data of thirty one patients with oral thrush were included in this study all having history immune compromise state e.g. diabetes, HIV sero-positives, malignancies with history of receipt of chemotherapy. Data was entered in Microsoft excel and proportions were calculated.*
Results: *Isolation of C. albicans was 74% and "non-albicans Candida" formed 26% of the Candida isolates. The commonest "non-albicans Candida" isolated were Candida tropicalis.*
Conclusion: *It is important to characterize the Candida, as the "non-albicans Candida" group are having high likelihood of being intrinsically drug resistant.*

Introduction

Candidiasis often presents as lesions in oral mucosa, commonly known as oral thrush. Oral thrush is usually an opportunistic infection associated with immune compromised states such as diabetes, extensive antibiotic usage, malignancies, chemotherapy and Human Immunodeficiency Virus [HIV] infection leading to Acquired Immunodeficiency Syndrome (AIDS).[1],[2],[3]

The commonest pathogen involved is Candida albicans. However, in the last few decades, the incidence of infections caused by "non-albicans Candida" species like Candida tropicalis, Candida parapsilosis, Candida glabrata and Candida krusei has increased, probably due to better techniques of speciation and isolation and also due to change in the profile of oral flora, due to medications. The recent emergence of Candida dubliniensis, C. haemolini and C. aurispose a serious threat to patient care, as these are resistant to commonly used antifungal drugs. This newly recognized opportunistic pathogen has been linked to oral candidiasis in HIV- infected and clinically diagnosed 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. [1],[2],[4]

Oral candidiasis can progress to oesophagitis, this interferes with adequate oral intake which in turn contributes to the general morbidity in these patients.[1] Although C. albicans remains the most frequent cause of oral candidiasis in AIDS patients, a number of reports have documented in infections caused by "non-albicans Candida" species such as C. tropicalis, C. glabrata, C. parapsilosis, C. krusei, C. lusitaniae and newer species like C. dubliniensis. These newly recognized opportunistic pathogens have 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. Non-albicans Candida are increasingly being isolated from oral lesions in patients admitted to pediatric ICU and patients receiving cancer chemotherapy. [2],[4],[5],[6]

Material and Methods

This study was undertaken to characterize the various species of Candida isolated in the department of Oral and Maxillofacial Pathology and Microbiology, in a tertiary care Medical and Dental College and hospital in Coastal South India, responsible for causing of oral thrush in patients with immune compromised state. Thirty one patients with oral thrush were included in this study all having oral thrush with a history of immune compromise state e.g. diabetes, malignancies with receipt of chemotherapy or HIV seropositives.

Two oral swabs each were collected from patients clinically presenting with oral thrush and cultured on Sabouraud dextrose agar without antibiotics [SDA] and SDA supplemented with antibiotics [50 µg/ml of Chloramphenicol and 5 µg/ml of Gentamicin] respectively. The sets of SDA tubes were incubated at 37^{o} C and room temperature respectively and examined every 48 hours till growth was obtained. Growth of yeast was confirmed by gram staining showing gram positive budding yeast cells with pseudohyphae.

These organisms were further characterised based on the following tests.[8],[9],[10]

[i] Germ tube test: A positive Germ tube test implied either C. albicans or C. dubliniensis. This was later confirmed by other tests.[8],[9]

[ii] Urease test: 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. [9],[10]

[iii] Growth on Chromogenic agar: Chrom-Agar for Candida (Hi Media, Mumbai, India) was prepared following manufacturer's instructions. By sub culturing from Sabouraud's dextrose agar plates, the Candida Hi Chrome plates were streaked and then incubated at 37^{o} C for 24- 48 hours. Colonies of C. albicans, C. tropicalis, C. parapsilosis, C. glabrata were identified using the Hi Chromemedia. Depending upon the color of the colony colour. Of the 31 strains, C. albicans were 23, they produced light green moist colonies on Candida Hi Chrome agar. C. tropical is strains produced a bluish colored colonies, C. parapsilosis produced cream colored colonies, C. glabrata colonies were pinkish blue colored while C. dubliniensis isolated from throat swabs in immune compromised patients produced dark green colonies.[11]

Results

In the present study 76% patients were males and in the age group of 21-50 years. 6% patients were elderly aged above 65 years of age.

Table 1: The patients with different diagnosis leading to immune compromised state is listed

S.no	Clinical Condition	Number
1.	HIV seropositives diagnosed as AIDS	11
2.	Diabetes mellitus (Uncontrolled/severe/ elderly patient)	11
3.	Tuberculosis (No other illness;HIVsero-negatives)	4
4.	Haematological and other Malignancies, H/O Chemotherapy	4
5.	Other immune compromised states	1
Total		31

Table 2: Characterization of Candida species isolated in the various patient samples

S.no	Isolate	Number	Percentage
1.	Candida albicans	23	74.1%
2.	Candida tropicalis	4	12.9%
3.	Candida glabrata	2	6.4%
4.	Candida dubliniensis	1	3.3%
5.	Candida parapsilosis	1	3.3%
Total		31	100

Statistical analysis: The data obtained was entered in Microsoft excel and proportions were calculated.

The most frequently isolated "Non-albicans Candida" isolated in our study was C. tropicalis (12.9%). followed by C. glabrata (6.4%), C. dubliniensis and C. parapsilosis 3.3% each.

Discussion

Oral thrush is a common clinical manifestation reported in the immunosuppressed individuals presenting/ referred in dental clinics. Orooesophageal candidiasis an AIDS indicator disease. In the present study, the isolation rate of Candida species in patients with oral thrush was characterised.[1],[3]

Various studies done by Korting et al have reported 74% isolation of C. albicans, while Gupta et al reported 50% isolation of C. albicans and Samonis et al reported 86% isolation of C. albicans from immune compromised patient groups. [2],[6],[7] These results were similar to the present study.

In our study the most frequently isolated "Non-albicans Candida" isolated in our study was C. tropicalis (12.9%). followed by C. glabrata (6.4%), C. dubliniensis and C. parapsilosis 3.3% each. Walmsley et al reported 20.6% isolation of "non-albicans Candida" from 97 HIV seropositive patients studied by them. McCreary et al 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 reported 6.5% of "non-albicans Candida"[1],[3],[12]

Most of the patients in the case reports and studies had an associated underlying disease leading to immune compromised state. Another common factor was that all had history of receipt of broad-spectrum antibiotics some time or the other in their lifetime, which may have been a contributory factor in the outgrowth of Non albicans candida species. Antitubercular drugs also lead to alteration of oral flora. McCreary et al reported 3/20 i.e. 15% of C. tropicalis from HIV seropositive patients while Walmsley et al obtained i.e. 2% C. tropicalis from HIV seropositive patients.[3],[12]

Conclusion

The increasing emergence of "non-albicans Candida" is a major cause of concern because of its ability to develop resistance to fluconazole. Also when they are missed 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. The morbidity and risk associated with oral thrush, along with an increase in refractory oral Candidiasis as well as the high incidence of Diabetes, HIV-AIDS, and rise of malignancy rates mandates that species identification and characterisation of Candida isolates should be carried out in most hospitals on routine basis.

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