ORIGINAL RESEARCH

Prevalence of Candida Species In Chronic Debilitating Patients With Oral Candidiasis- A Pilot Study

Aparna. K,[1] N. Vezhavendhan,[2] Pramodhini[3]

ABSTRACT

Introduction: Oral candidiasis is a common opportunistic infection caused by an overgrowth of candidal species. The presence and growth of candida in the oral cavity is often said to be increased in patients with many systeic diseases. The accurate identification of strains isolated from such patients are important since they are more likely to carry species other than *C. albicans*, which might not be sensitive to certain antifungal agents. This study was carried out to determine the different Candidal species in chronic debilitating disease.

Materials and methods: Chronic debilitating patients with Oral Candidiasis were taken as study group. The swab was taken from lesional area and inoculated in Sabouraud's Dextrose Agar culture medium for 48 hours followed by sub-culture in Chromagar candida for candidal species differentiation, the results were statistically analyzed using Chi-square test (Kendall's W test).

Results: The study of the different Candidal species grown in the cultures of the smears taken from the study group was recorded. This included clinically evident oral candidial infection in patients suffering from diabetes mellitus, Chronic pulmonary lesions, Chronic renal and liver disorders. The study result showed the growth of non-albican species like Candida dubliniensis in patients with diabetes and chronic pulmonary disease. The growth of Candida krusei was recorded in patients with chronic renal disorder.

Conclusion: The accurate identification of Candidal strains may be a useful tool to provide appropriate antifungal therapy especially for drug resistant Candidiasis.

Key words: Candida species, Candidiasis, Chronic debilitating patients, Chromagar culture.

How to cite this article: Aparna. K, N. Vezhavendhan, Pramodhini. Prevalence of candida species in chronic debilitating patients with oral candidiasis- a pilot study. Int J Cont Med Res. 2015;2(1):32-38

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Source of Support: Nil

Conflict of Interest: None

INTRODUCTION

Candida is the commonest fungi causing candidiasis in humans affecting mucosa, skin, nails and organs of the body which was first isolated in 1844 from the sputum of a tuberculous patient. Most of the species of candida have shown to exhibit similar features at the macroscopic and microscopic level..[1] Twenty species of candida are considered to be significant pathogens causing various infections in humans. Most important opportunist endogenous infection producing candida species are C.albicans, C. tropicalis, C.krusei, C.glabrata, C. guilliermondii, C.parapsilosis, C.kefyr, C.lusitaniae, C.dubliniensis, C.viswananthi, C.stellatoidea.[2]

Candida albicans which is the most common cause for oral candidiasis is also the most virulent and pervasive of all candida species.[3,4,5] Various virulent factors like adhesion, enzyme production, hyphal formation, phenotypic switching and thigmotropism contribute to the pathogenicity of the species.[6] However, its increased adherence to mucosal surfaces is shown to be a prerequisite to successful colonization and subsequent infection. Previous studies have

shown interspecies variation in candidal adhesion to buccal epithelial cells with the albicans demonstrating the greatest adhesion. Candida grow at different environmental conditions and form microbial communities called "biofilms" by attaching to the host surface.. Non-albicans candida species are also found along with the albicans in these polymicrobial biofilms and thus an extensive interspecies interactions may take place in these adherent populations.[7] These characteristics of candida when superimposed with factors like aging, xerostomia, antibiotic therapy, smoking, malignancies, immune deficiencies, chronic debilitating diseases may cause candidiasis.

The rising incidence of candidiasis especially in patients with chronic debilitating diseases is causing concern with serious problem worldwide.[8] Candida species not only differ in their virulence properties, their susceptibility to antifungal drugs also differ. The newer emerging species of candida tend to be resistant to routinely administered antifungal agents like fluconazole, ketoconazole and render them ineffective, sometimes causing undesirable side effects.[9] Such patients may need either prolonged treatment or altered therapy with combination of drugs. Hence recognition of different species of candida has a significant role in treatment plan and prognosis of the disease. The present study was carried out to find the prevalence of albicans and non-albicans in chronic debilitating diseases and to evaluate the role of non-albicans candida species in treatment regimen.

MATERIALS AND METHOD

The present study was conducted with the approval from Institutional Ethical Board and informed written; signed consent was taken from all the patients. The study was carried out on 6 varying groups of chronic debilitating patients who had oral candidiasis that included patients with uncontrolled diabetes; patients with diabetes associated with other long standing systemic disease –[Table-2] 3 samples taken from patients who also had complaints of either long standing pulmonary disease or some with cardiac ailments, under medication; those suffering from chronic pulmonary disease; patients suffering from chronic renal diseases; those with chronic liver disorders, and those patients who were on immunosuppressive medication. The patients who were under treatment for any of these long standing conditions and reported to our institution for treatment were considered and examined for oral lesions. The sample size calculation was done with previous referred studies.[10]

The swab was collected from the lesion area in the oral cavity from the patients of these groups. The groups were divided depending on the chronic diseases they were suffering from, with diabetic patients having uncontrolled blood glucose level were assessed with HbA1c values greater than 8.5% as uncontrolled, whereas patients suffering with other associated chronic diseases were considered with the chronicity of the condition where the patients were diagnosed to have chronic renal or liver disease or pulmonary dieases for more then 2 years or so and were under medication. The groups were thus divided as shown in the table-2. The patient were suffering from any of these chronic illness and showed the signs of oral candidiasis. After the examination of such lesions, the swab taken from the lesional area was sent for microbiological examination-culture and fungal growth was observed and assessed. (Fig 1). It was first cultured on Sabouraud's Dextrose Agar (SDA) for 48 hours. After the confirmation of fungal growth, germ tube test was done. Germ tube test helps differentiate albicans from non albicans due to its ability to produce short, slender, tubelike structures called germ tubes when incubated at 35°C for 2 to 4 hours in pooled human serum.[11,12] The clinical material was then sub-cultured on Chromagar candida for species identification (Fig.2).

The clinical material cultured on sabouraud's dextrose (glucose) agar [with chloramphenicol] at room temperature of showed a cream coloured (curdy white), smooth, pasty growth having a yeasty odour confirming the fungal growth. Species identification of candida isolates was performed by subculture of the obtained colonies in Chromagar Candida (CaC). It is a selective and differential chromogenic medium for rapid screening and identification of different Candida species. Chromagar Candida contains various substrates for the enzymes of yeast species. Beta–

N-acetylgalactosaminidase produced by C.albicans enables the chromogenic substrates to be incorporated into the media. This media enables different species of candida to be expressed in different colours and thus the identification of different species. (Table-1)
The patients with chronic debilitating diseases were categorized into following six groups: (Table-2)

1. Diabetics,
2. Diabetics associated with other long standing systemic disease,
3. Chronic pulmonary diseases,
4. Chronic renal disease,
5. Chronic liver disorder,
6. Immunocompromised patients.

Depending on the obtained result, these sampled groups were categorized into

1. Samples showing C.albicans
2. Samples showing non-albicans growth
3. Samples showing no growth.

STATISTICAL ANALYSIS

The prementioned groups with the obtained result were subjected to the statistical analysis using Chi-square test (Kendall's W test).

RESULTS

Out of the total 25 samples collected, 17 showed albicans, 3 of them were non-albicans and 5 had no fungal growth. Though the majority 68% of samples showed albicans growth, 12% of samples showed the growth of non-albicans species. The non-albicans species detected included C.dubleinensis and C.krusei. Fortyeight percent of samples were taken from diabetic patients and all showed albicans growth. The debilitating disease most affected with non albican species was the chronic pulmonary disease and chronic liver disorder in this study, p value < 0.001 (Table 3 & 4).

DISCUSSION

Though the albicans and non-albicans species of candida have many similar features, their epidemiology, virulence characterisics and anti-fungal susceptibility show variations.[13,14] Inspite

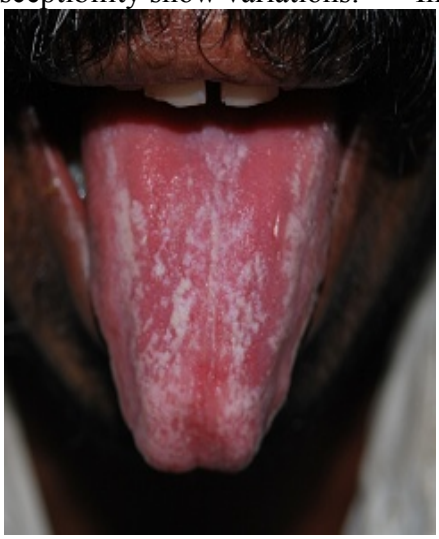

Figure-1: Clinical picture of a patient with oral candidiasis

of these similarities and differences in their characterisics, they produce similar disease condition which varies in their severity from oral thrush to invasive states of disease and also in their antifungal susceptibility.
Candidal antigens are those derived from its cell wall components and those in the cytoplasm.[15] Cell wall Antigens is based on agglutination reactions that use antisera to the components of the cell wall. Mannan is the major antigenic components of the candida cell wall. The glucan polymers which are in greater abundance than mannans in the C.albicans cell wall is immunologically less active. So cell wall of C.albicans is not antigenically consistent. Cytoplasmic Antigens have not yet been associated with specific cytoplasmic components of the cell wall. Delayed hypersensitivity to candida is used as an indicator of functional integrity of cell mediated immunity.
Compared to the non-albicans species, Candida albicans shows more adaptability as an oral commensal, at its cellular and molecular level. The main contributing factor, among others, for their virulence is the adhesion to the epithelial surface. Decreased adhesion of non-albicans species has been suggested to contribute to their lower virulence. This limits their ability to cause disease in healthy individuals. Compromised hosts are prone to array of opportunistic infections. Hence Oral Candidiasis is widely recognised among immunocompromised and


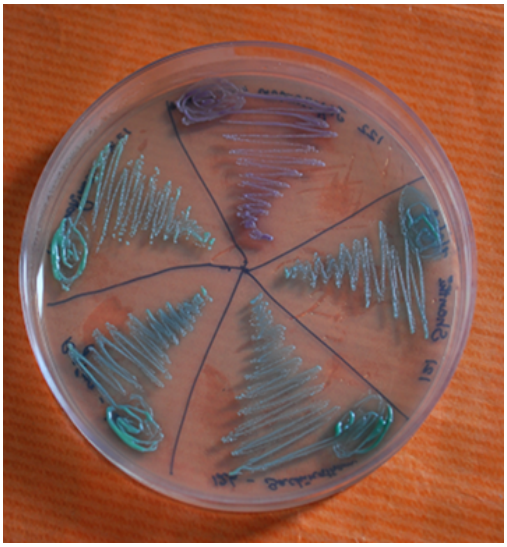

Figure-2: Chromagar plate showing candida growth

Colony color on Chromagar candida	Candida species identification
Light green	C. albicans
Dark green	C. dubliniensis
Fuzzy, rough, large pink	C. krusei
Pale edges on dark pink (purple)	C. glabrata
Dark blue with halo	C. tropicalis

Table – 1: showing the colour of the colonies of different species of candida when cultured in Chromagar medium.

		Debilitating diseases						Total
		Diabetics	Diabetics associated with other long standing systemic disease	chronic pulmonary diseases	chronic renal disease	chronic liver disorder	Immunocompromised patients	
Distribution of group	Albicans	9	2	4		1	1	17
	Non Albicans		1	1	1			3
	No Growth					1	4	5
Total		9	3	5	1	2	5	25

Table -2: Describes the cross tabulation that compares the distribution group with that of the debilitating diseases

Ranks (Kendall's W Test)	
	Mean Rank
Debilitating diseases	1.80
Distribution of group	1.20
P<.0001	

Table -3: Statistical analysis assigning the mean rank to each study group.

Test Statistics	
N	25
Kendall's W(a)	.600
Chi-Square	15.000
df	1
Asymp. Sig.	.000
a Kendall's Coefficient of Concordance p<.0001	

Table -4: Statistical analysis

chronic debilitating patients. Candida albicans is the most dominant species causing infection. In the present study, 68% cases showed Candida albicans and the non-albican species reported in the present study sample included C.dubliniensis and C.krusei.

In the previous studies on diabetic patients, Khaled H Abu et al (2006) in Amman, Jordan studied 132 diabetic patients and reported 81.8% albicans growth with non-albicans strains reported to be C.tropicalis, C.parapsilosis and C.glabrata,[16] Willis et al in 2000 had 63.2% of diabetics with albicans infection &, C.dubliniensis and C.glabrata being the non-albicans species reported,[17] F. Z. Aly et al in 1995 reported with 43% of diabetics with albicans.[18] In their study, non-albican species detected were C.glabrata and C.tropicalis. Our study results on diabetic patients had 92% with albicans infection. The non-albican species reported was C.dubliniensis.

In the literature review of the studies done on candida infection in tuberculosis/ pulmonary diseases, Sehar Afshan Naz and Parween Tariq in 2004 conducted a study in Karachi on 500 tuberculous patients and reported that 55% of their study subjects having infection with C. tropicalis,[19] Silvia Maria Rodrigues Querido et al in 2011 reported with 42.5% albicans and rest being C.tropicalis and C.glabrata,[20] Latha. R et al in 2011 used sputum specimens of 721 tuberculous patients in their study conducted in Pondicherry, India, also had similar result with around 55% of non-albicans species of C.tropicalis, C.glabrata, C.parapsilopsis, C.krusei.[21] Jain S K et al in 1982 reported a comparatively higher incidence of albicans with a prevalence of non-albicans around 20% with the prevailed species being C.tropicalis, C.pseudotropicalis, C.krusei.[22] The present study showed albicans prevalence of 71.43% and the non-albicans species reported to be C.dubliniensis.

Thus, recent studies have shown that in chronic debilitating patients, the proportion between albicans and non-albicans species is more balanced as found in a previous study done by Shaheen MA and Taha M in 2006.[10] Detection of non-albicans species considered to be of low virulence may be the result of decreased host defenses and environmental exposure to those species. Presence of non-albicans species can also result from frequent use of antifungal therapy in the compromised patients as they are associated with strains that are resistant to conventional antifungal therapy. Lyon et al. in their study observed that 2 μg/ml of fluconazole inhibited the growth of ninety percent of C.albicans, C. parapsilosis, C.tropicalis with all the isolates of C.krusei showing resistance at this concentration of fluconazole..[23] Hence fluconazole can be used as drug of choice for the susceptible species, but for C.krusei and C.glabrata, voriconazole can be used as drug of choice in step-down therapy. In recent years, newer group of antifungals like echinocandin have been used to replace azoles and polyenes. Echinocandin inhibits the enzyme D-glucan synthase, which is needed for the synthesis of fungal cell wall. This enzyme is absent from mammalian cells thereby reducing potential host cell toxicity.[24,25] Therefore, it is evident from the present trends that choice of antifungal for an unknown Candida species should be based on the identification of the strain or species of Candida for an efficient treatment management.

CONCLUSION

Identification of species and differentiation between them is important for successful clinical management. Numerous risk factors for invasive Candida infection have been reported and several antifungals are widely available, the optimal management of candidiasis in chronic deblitating

patients remains a challenge. The choice between prophylactic, empirical and pre-emptive therapy is crucial. Though, this study was limited to species identification, the need for regular investigations into antifungal resistance could further enhance the importance of such a study in management of one of the most commonly encountered infections in these chronic debilitating patients, thereby emphasising on a constant need for research into new and effective agents to treat oral candidosis.

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