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Original Article

Prevalence of *Candida* among Denture Wearers and Nondenture Wearers

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ABSTRACT

Introduction: A denture wearer is highly prone to develop *Candida*-associated denture stomatitis (CADS) due to the conversion of the normal oral commensal *Candida* spp. into a pathogen under favorable conditions. Immuno-compromised status, trauma from the prosthesis, other systemic conditions, and improper maintenance of the dentures by the patient are few of the causative agents which turn the oral balance into an unhealthy and unsuitable foundation for the wearing of the prosthesis. **Objective:** The objective of this study was to compare subjects wearing complete dentures and nondenture wearers regarding *Candida* isolates associated with disease and colonization among the different age groups. **Methodology:** A cross-sectional study was conducted among 60 subjects, 30 wearing complete dentures (Group-A) and 30 nondentures wearers (Group-B), were matched for gender, race, and age. The unstimulated saliva sample was collected and cultured in CHROMagar using standard protocols. **Results:** The mean value of the candidal colony-forming units with respect to Group A is *Candida Albicans* 0.36 ± 2.008, *Candida krusei* 0.27 ± 1.061, *P* value Group B: *C. albicans* 0.73 ± 2.196, *C. krusei* 0.36 ± 1.084. There was a significant relation between denture wearers and heavy growth of *C. albicans* and *C. krusei* in saliva culture with a *P* value of *P*-0.054 and *P*-0.036, respectively. **Conclusions:** These results indicate that denture wearers with oral *Candida* had a higher prevalence of CADS. Patients with removable prostheses should be informed about the importance of proper prosthesis and personal hygiene since dentures and age-related immunosuppression are both well-known risk factors associated with candidiasis development, the presence of yeast, even in healthy denture wearers, should be considered a risk factor for denture stomatitis that increases with the duration of denture use.

KEYWORDS: *Candida, denture stomatitis, denture wearer*

INTRODUCTION

Infections due to *Candida* species and other fungi have increased dramatically in recent years and are of prime importance because of the rising number of immunocompromised patients.[1] Although *Candida albicans* remains the most common cause of infection, the frequency attributed to other members of the genus is also increasing. This is due to the increase in the number of at-risk individuals, particularly those with impaired immunity such as transplant recipients, cancer patients receiving chemotherapy, and human immunodeficiency virus (HIV)-infected patients.[2]

In the 1980s, *C. albicans* accounted for more than 80% of all Candidal isolates recovered from nosocomial yeast infection. Of late, the emergence of *Candida* species other than *C. albicans* is a matter of concern in several major institutions. These species are also shown to have reduced susceptibility to antifungal agents.[3] The frequency of isolation of *Candida krusei*, *Candida*

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glabrata, *Candida tropicalis*, and *Candida parapsilosis* is steadily increasing globally. In the oral cavity, *Candida* is a normal commensal along with other microbiota. In an immunocompromised patient who is wearing a complete denture, there is a risk of development of *Candida*-associated denture stomatitis (CADS). This is due to the conversion of the normal commensal to an infection-causing pathogen. Various factors enhance the development and progression of the disease resulting in CADS.[3] The prosthesis acts as a foci and trauma from the denture will facilitate infection. Several species of *Candida* have known to be involved in the process. Even though *C. albicans* is the most frequent species, recently, non*albicans* species have been reported to be dominant. Hence, identification of the causative species is essential for the rapid treatment initiation with an appropriate antifungal agent.[4] There are many techniques to identify *Candida* species, but among them a technique using CHROMagar *Candida* (HiMedia, Mumbai) can easily identify different species of *Candida* on the basis of colony color and morphology.

This study was done to compare subjects wearing complete dentures and nondenture wearers regarding *Candida* isolates associated with disease and colonization.

Methodology

Sampling

A cross-sectional study was conducted among 60 subjects, 30 wearing complete dentures (Group-A) and 30 nondentures wearers (Group-B), were matched for gender, race, and age. The unstimulated whole salivary samples were collected 2 h after any oral or visual exposure to foodstuffs. All the salivary samples were collected in sterile cryovials between 10 a.m. and 11 a.m. to prevent any bias in the concentration of the saliva due to circadian rhythm.

Inclusion criteria

1. Age group of 50–70 years, male or female patients
2. Patients wearing complete denture prosthesis
3. Patients wearing the prosthesis for more than a year
4. Patients not on any antifungal medication
5. Patients who are willing to participate in the study.

Exclusion criteria

1. Age <50 and more than 70 years
2. Patients not wearing complete denture prosthesis
3. Patients wearing the prosthesis for less than a year
4. Patients on antifungal agents
5. Patients who are not ready to participate in the study.

All the patients were given a uniform controlled diet from their central kitchen 1 day before the collection of saliva for investigation so as to rule out any bias in the results.[5-7] All the patients were brought in for saliva collection 2 h after the morning breakfast. The unstimulated whole salivary samples were collected 2 h after any oral or visual exposure to foodstuffs. All the salivary samples were collected in sterile cryovials between 10 a.m. and 11 a.m. to prevent any bias in the concentration of the saliva due to circadian rhythm.

Preparation for saliva collection

1. Plastic drinking straws were cut into 2-inch (5 cm) pieces
2. For each patient, one straw piece and a cryovial was given
3. Patients were asked to rinse their mouth with water 10 min before the collection of saliva samples.

Collection of saliva

1. Patients were instructed to imagine eating their favorite food and allow saliva to pool in the mouth
2. With the head tilted forward, patients drool their saliva through the straw and saliva was collected in a cryovial.

Immediately after the collection of saliva, it was stored at a temperature of 4°C using dry ice pack in cello chiller until it was transported to the laboratory.

Chromogenic agar culture

Each isolate was cultured on Sabouraud Dextrose Agar at 30°C for 48 h. After this, they were seeded on CHROMagar™ *Candida* (CHROMagar microbiology) and incubated at 30°C for 48 h. The CHROMagar™ allows selective yeast isolation, identifying colonies of *C. albicans*, *Candida dubliniensis*, *C. tropicalis, and C. krusei* by morphology and color reaction. The strains were identified according to the manufacturer's instructions, which define *C. albicans* or *C. dubliniensis* as green colonies, *C. tropicalis* as steel blue colonies, *C. krusei* colonies as showing rose color and rough aspect, and the other species as developing colonies from white to rose.

Results

Among the 30 study group, 14% (n = 7) of the patients were vegetarians and 86% (n = 23) were nonvegetarians. In the 30 controls, 22% (n = 11) of the controls were vegetarians and 78% (n = 39) were nonvegetarians (P = 0.298).

The mean value of the candidal colony-forming units with respect to Group A is *C. albicans* 0.36 ± 2.008, *C. krusei* 0.27 ± 1.061, P value group B: *C. albicans* 0.73 ± 2.196, *C. krusei* 0.36 ± 1.084. There was a significant relationship between children with special

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needs and heavy growth of *C. albicans* and *C. krusei* in saliva culture with a *P* value of *P*-0.054 and *P*-0.036, respectively [Table 1].

Discussion

Denture stomatitis has been found to be a common problem associated with denture wearing. The causative agents for this have been ill-fitting or poorly-fitting complete denture prosthesis causing trauma to the underlying mucosa or improper maintenance protocol by the wearer.[8-10] In addition, if the patient has suppressed immunity as in diabetes or HIV, the chances that the normal oral commensal, *Candida* may convert itself into a pathogen to cause CADS. Approximately 65%–70% of the denture-wearing population has been identified as afflicted with CADS in other parts of the universe.

C. albicans has been the most common causative species in CADS. However, recently it has been reported that there is a shift in this aspect and other species have been found to be superseding *C. albicans* in causing the infection.[11,12] This is due to the development of drug resistance or mutations in the strains causing change in the phenotype of the microorganism. In a clinical situation, rapid species-level identification is very essential as it has substantial impact on the treatment decisions.

This study was done to assess the prevalence of CADS and also to identify the most frequent causative species related to the condition. Different media have been used to carry out the speciation procedure. Among them, CHROMagar has been found to be rapid, accurate, and economical. This medium contains a chromogenic β-glucosaminidase substrate which releases differently colored compounds on degradation by the specific enzymes released by the species. In this medium, identification of the species is done by the development of different colors. *C. albicans* (light green), *C. tropicalis* (steel/light blue), *C. krusei* (white fuzzy), and *C. glabrata* (light pink).[13,14] In the present study, it was identified that *C. albicans* is the most prevalent (50%) species followed by *C. tropicals* (33%) and *C. glabrata* (18%).

Table 1: Prevalance of candidal growth in chromagar

CHROMagar	Groups	Mean±SD/cfu	*P*
Candida albicans	Group I	0.36±2.008	0.054
	Group II	0.73±2.196	
Candida krusei	Group I	6.57±11.61	0.136
	Group II	6.6±7.784	
Candida tropicalis	Group I	0.03±0.1825	0.317
	Group II	0	
Candida glabrata	Group I	0.07±0.365	0.981
	Group II	0.03±0.183	

SD: Standard deviation

According to many authors, *C. albicans* has been the most frequent species of *Candida* in causing denture stomatitis[15-17] Cannon *et al*. are of the opinion that this yeast has the ability to colonize different areas of the oral cavity due to the specific interactions between the two. It has been known to adhere even to complement receptors and some sugar residues that are present on the host surfaces. Tissue invasion by this species causes infection of the oral mucosa.

Silva *et al*. said that *C. albicans* can not only adhere to the oral mucosa but also can colonize the surface of the acrylic denture when it is poorly maintained. The biofilm on the denture can enhance its adhesion adding to its virulence. Wearing of a denture has been found to[18-27] enhance adhesion of *C. albicans* by Witzel *et al*. in the year 2012. However, Williams and Lewis are of the opinion that even though *C. albicans* is the dominant species, and it is the problem of the emergence of non *C. albicans*, Samaranayake *et al*.,1994 studied the virulence and pathogenicity of *Candida* species and stated that *C. krusei* predominately emerged as a notable pathogen with a spectrum of clinical manifestations such as fungemia, endophthalmitis, arthritis, and endocarditis, most of which usually occur in compromised patient groups in a nosocomial setting. Several researches have also discovered that *C. krusei* has been recognized as a potentially multidrug-resistant fungal pathogen due to its intrinsic fluconazole resistance combined with reports of decreased susceptibility to both flucytosine and amphotericin B. Given the variable activity of voriconazole against this species, and testing of the susceptibility of *C. krusei* to these potentially useful antifungal agents may be warranted to help guide therapeutic decisions.

Conclusions

- These results indicate that denture wearers with oral *Candida* had a higher prevalence of CADS
- Patients with dentures and age-related immunosuppression are both well-known risk factors associated with candidiasis
- The presence of yeast, even in healthy denture wearers, should be considered a risk factor for denture stomatitis that increases with the duration of denture use
- It is high time to inform the denture wearers regarding the importance of proper prosthesis maintenance and oral care to prevent denture-associated candidiasis.

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Conflicts of interest

There are no conflicts of interest.

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