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Candida Associated Denture Stomatitis

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ABSTRACT

Objectives- Denture stomatitis (DS) is a common lesion that affects denture wearers. The purpose of the study was to compare the prevalence and species of Candida in the oral cavity of dentate and denture wearers and to study their biofilm formation in relation to denture stomatitis.

Material and methods- Oral rinse samples obtained from 25 healthy male complete denture wearers and 25 dentate subjects were microbiologically investigated for growth of Candida. Isolated Candida species were identified by conventional methods and confirmed by using automated identification system Vitek 2 Compact (bioMerieux). Biofilm forming property of each strain was detected by using microtitre plate method and its relation to denture stomatitis was studied.

Results- Candida spp. were isolated from the oral cavities of 32% dentates and 48% denture wearers (p-value=0.248; non-significant). C. albicans was the most common species isolated from both the groups. 10% of the strains showed biofilm forming characteristic. Denture stomatitis was observed in 20% of denture wearers and Candida was isolated from all these subjects.

Conclusions- Both C. albicans and non-albicans species of Candida are associated with oral carriage suggesting that non- albicans Candida spp. should also be taken into account for the maintenance of oral healthy mucosa. Candida colonisation is responsible for causing DS and biofilm formation is an important virulence trait during candidosis.

Introduction

Candida infections are common and represent a significant clinical problem in oral cavity (1). In healthy, dentulous persons Candida rarely causes disease while in complete denture wearers, its proliferation in the space between the maxillary denture and the palatal mucosa gives rise to denture stomatitis (DS) in about 50-60% (2). Adherence followed by colonisation and subsequently biofilm formation have been thought to be responsible for denture related stomatitis (3). Candida species are known to produce biofilms which are structured microbial communities attached to a surface and encased in a matrix of exopolymeric material (4). These are the survival mechanism to ensure residence in the mouth. Also, Candida present in the oral cavity serves as a reservoir for inoculation and infections elsewhere in the body. When it penetrates the epithelium and invades the host tissues, this may lead to blood-stream infections and systemic infections which are difficult to treat and are associated with high mortality. Therefore in the present study we focussed on the Candida species isolated from the oral cavity of dentate and denture wearers and studied their biofilm formation in relation to denture stomatitis.

Material and methods

A total of 50 apparently healthy male subjects (25 dentates and 25 complete denture wearers) were enrolled in the study. The persons who were taking antifungal agents or antiseptic mouth washes, any medication known to predispose to oral candidosis, or who had a medical history such as diabetes mellitus or anaemia that could predispose them to oral candidosis or promote candida carriage were excluded from the study. Intra oral examination was conducted for diagnosis of denture stomatitis after the subjects had removed their dentures. The specimen was collected by asking the subjects to rinse with 10 ml of Phosphate buffered saline (PBS) for 60 seconds. The rinse sample was centrifuged at 3500 rpm for 10 minutes and the deposit was divided into two portions: one was used for KOH prepration for direct visualisation of budding yeast cells and the other was inoculated on Sabouraud's Dextrose agar and incubated aerobically at 37°C for 48 hours (5). Lactophenol Cotton Blue mount showing budding yeast cells from the colonies obtained were identified by conventional methods such as germ tube test, sugar fermentation and assimilation reactions. Further confirmation of Candida species was done by using automated identification system 'Vitek-2 Compact' (bioMerieux).

Biofilm forming property of the isolated Candida strains was detected as described by Shin et al (6). Biofilm production by each isolate was scored as negative (%Transmittance bloc, < 5), 1+ (%Tbloc, 5 to 20), 2+ (%Tbloc, 20 to 35), 3+ (%Tbloc, 35 to 50), or 4+ (%Tbloc, >50). Each isolate was tested in duplicate. C. albicans ATCC 90028 and C. parapsilosis ATCC 96142 were used as controls.

Results

Out of 25 dentates and 25 denture wearers, Candida spp. were isolated from the oral cavities of 8 (32%) and 12 (48%) subjects respectively (p-value=0.248; non- significant). Distribution of various species of Candida is shown in Table 1. C. albicans was the most common species isolated from both the groups but statistically the difference in the isolation of C. albicans and non-albicans Candida species was not significant (p-value= 0.142, Table 1).

Study of the biofilm formation showed that only two of the 20 (10%) isolated strains of Candida had biofilm forming property and both these strains were C. albicans. One of these strains was isolated from dentate group and was quantified as 1+ and the other which was isolated from denture wearer group as 3+ for biofilm formation.

On oral examination denture stomatitis was observed in 5 (20%) denture wearers and Candida was isolated from all these subjects. Species associated with DS were C. albicans (2), C. tropicalis (1), C. glabrata (1) and C. krusei (1).

Discussion

Candida has been recognised as a part of the normal flora and its prevalence in oral cavity varies between 15 to 75% (7). This facilitates its encounter with most implanted denture materials which can become contaminated prior or during implantation. Hence changes in the oral environment effected by certain factors such as denture wearing can cause changes in the oral microflora. In our study the prevalence of oral Candida was 48% in denture wearers and 32% in dentates. Arirachakaran et al observed the prevalence of oral Candida in 85% and 77.5% denture wearers and dentate subjects respectively (5). Darwazeh AM et al reported 60% prevalence in denture wearers (8). The low prevalence of Candida in our subjects could be because of the geographical variation or the better dental hygiene of the population studied. Although the detection rate of Candida was higher in complete denture wearers as compared to dentate group in our study, the difference was statistically insignificant (p-value=0.248). This affirms the results from earlier studies (5).

In the present study C. albicans was the most common species recovered from both the groups. The prevalence was 41.6% in denture wearers and 75% in dentate group. Vanden Abbeele et al (9) and Arirachakaran et al (5) had also reported C. albicans as the most common species and others like C. glabrata and C. tropicalis as the second most prevalent species in healthy denture wearers respectively. However in our study C. krusei was the second most common species followed by C. glabrata. Isolation of the large number of non- albicans Candida species is important as it may be related to the prophylactic use of antimycotics such as azoles. These species are relatively more resistant to azoles and thus make the infections caused by them difficult to treat.

Denture stomatitis is a frequent finding among denture wearers. In our study DS was observed in 20% denture wearers and Candida was isolated from all these subjects. It has been reported that the yeasts could be demonstrable in 78-100% of patients with denture-induced stomatitis (10). Rabelo et al reported Candida isolation in 97.3% of the patients with DS and suggested that the high prevalence of colonisation of Candida in patients with DS collaborates with long term use of denture which positively influences the colonisation and predisposition to installation of DS (1). Various species of Candida associated with DS in our study were C. albicans (2), C. tropicalis (1), C. glabrata (1) and C. krusei (1). Other authors have also reported the predominance of C. albicans followed by non albicans Candida spp. such as C. krusei, C. glabrata and C. tropicalis in cases of DS (1).

We also studied the biofilm formation in various Candida isolates and it was observed that this characteristic was present in two out of 20 (10%) isolates of our study; one from each group (denture wearers and dentates). Both these isolates were C. albicans. Other authors have reported biofilm positivity in 39-64% of their isolates (6,11,12). Dag et al had also found that C. albicans had larger percentage (39.3%) of biofilm positivity in comparison to non-albicans Candida species (37.79%) (11). However, Shin et al (6) and Muni et al (12) reported non-albicans Candida spp. to be more biofilm producers than C. albicans. Since ability to form biofilm is associated with the ability to cause infection, resistance to antifungal agents and protection from host defences, its presence in Candida isolates from dentate group suggests that if denture is required in near future it could become the source of denture stomatitis.

Within the limitations of the present study, the following conclusions could be drawn;

1. C. albicans and non-albicans species of Candida are associated with oral carriage in both the denture wearers and dentates. Presence of non- albicans spp. of Candida suggests that non- albicans Candida spp. should also be taken into account for the maintenance of oral healthy mucosa as these yeasts are usually azole resistant and infections caused by them are difficult to treat.
2. As Candida colonisation was found to be associated with all the cases of DS, controlling Candida through appropriate denture management could help promote general oral health and assure the prevention of oral diseases.
3. As biofilm formation is considered as an important virulence trait during candidosis, the next logical step of the study should be to focus on in- vivo grown biofilms, mixed bacterial- fungal biofilms and use of new materials and other preventive measures that could be employed to inhibit biofilm formation and thus DS.

Table 1 DISTRIBUTION OF VARIOUS CANDIDA SPECIES ISOLATED FROM DENTURE WEARERS AND DENTATES

Group	C.albicans[c]	Non albicans Candida[d]				
		C. tropicalis	C. glabrata	C. dubliensis	C. krusei	Total
Denture wearer (n= 12)[a]	5	1	2	1	3	7
Dentate (n= 8)[b]	6	1	1	-	-	2

n= no. of subjects positive for Candida

Statistical analysis-

a & b p-value = 0.248 (not-significant)

c & d p-value = 0.142 (not-significant)

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