Assessment of Candida species colonization and denture-related stomatitis in complete denture wearers

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Candida species are considered the primary causative agents of denture stomatitis, but their role in colonization and disease in denture wearers remains undefined. In this study, we investigated risk factors associated with progression to Candida-related denture stomatitis in patients using complete dentures, and we genetically identified Candida isolates associated with disease and colonization. We recruited 114 retirement home residents for this study, from whom oral mucosa samples were collected and cultured following oral cavity exams. Morphologic analysis was used to identify potential yeast-positive cultures, which were then characterized further by RFLP analysis. C. albicans was the most frequently recovered species (61; 41.5%), followed by C. glabrata (27; 18.4%), and C. tropicalis (19; 12.9%). In addition, 16 isolates (10.9%) of C. dubliniensis were recovered, which was the first identification of this species in clinical samples from Iran. This study demonstrated a significant association between the duration of denture wear and oral candidiasis. Furthermore, we noted a high prevalence of C. dubliniensis in complete denture wearers.

Keywords Candida, candidiasis, denture wear, stomatitis, oral microflora

Introduction

Dentures affect the nature of the oral cavity microenvironment [1]. Alterations to the oral mucosa can result from denture-mediated mechanical irritation, or inflammatory responses induced by denture-related materials [1,2]. In addition, biofilm formation on denture surfaces, accompanied by subsequent allergic reactions resulting from microbial colonization and/or their secreted metabolites, further affects the nature of the oral microenvironment [3]. Candida species, which comprise 25–50% of the oral cavity microbiota of healthy individuals, are one of the main causes of microbial biofilm formation on catheters and dentures, and comprise 80% of the organisms recoverable from the oral mucosa of denture wearers [3,4]. Although Candida albicans is commonly associated with denture use, other Candida species such as Candida glabrata are frequently recoverable from dentures and underlying mucosal tissues [4]. Candida colonization and biofilm formation on dentures can be further affected by the species of colonizing Candida, oral hygiene practices, and denture characteristics [5,6]. Personal hygiene, such as denture removal at night, denture cleanser use, and smoking have been shown to affect colonization and denture-associated stomatitis [2,6–8]. Denture-related structural factors, including vertical dimensions, material integrity, and fit, affect yeast colonization and subsequent denture-related stomatitis [6,9,10]. Stomatitis has been reported in more than 60% of denture wearers, and although it is typically asymptomatic, it is associated with leukoplakia, pseudomembrane (thrush) formation, erythema, and angular cheilitis [11].
In this study, we assessed the prevalence of denture stomatitis in complete denture wearers and its association with particular species of colonizing Candida. In addition, the influence of risk factors on Candida colonization and denture stomatitis was evaluated.

Materials and methods

Study demographics and sample collection

Institutionalized individuals (n = 114) from four retirement homes from Shiraz, Iran and 53 individuals from a retirement society (Kanone Jahandidegan) were enrolled in this study. Their ages ranged from 43–103 years and their average and median ages were 70.4 and 71 years, respectively. All participants had complete dentures for both the mandible and the maxilla. After examination of the oral cavity, denture specimens were collected by swabbing the oral mucosa or lesions (if present), as well as internal denture surfaces after patients rinsed their mouths with tap water. All participants provided written informed consent approved by the ethics committee of Shiraz University of Medical Sciences. Information relating to patient demographics including age, gender, drug use history, smoking habits, stomatitis symptoms, in addition to factors relating to denture wear, such as how long respective patients had used dentures, cleaning methods used, daily use frequency and vertical dimensions were collected in questionnaire forms by study dentists. Individuals receiving antifungal or antibacterial antibiotics (as well as individuals wearing partial dentures) were excluded from the study.

Samples were inoculated onto Sabouraud dextrose agar plates (Merck, Darmstadt, Germany) containing 0.01 g chloramphenicol (Fluka, Steinheim, Switzerland). Plates were incubated at 31°C for 2 weeks and examined frequently for developing colonies. The plates were then scored based on the number of colonies identified and respective colonies were then subcultured and purified on Sabouraud dextrose agar and Chromagar Candida (CHROMagar, Paris, France) plates. Isolates were stored at −20°C until further analyzed.

Characterization of Candida species

Isolated yeast colonies were screened according to their physiological properties such as germ tube test, ability to produce chlamydoconidia and color formation in Chromagar Candida Medium [12]. In addition, genomic DNA was extracted and purified using glass bead preparation as described [13] and Candida species were identified by amplification of the ITS1-5.8S-ITS2 rRNA gene regions and amplified PCR products digested with MspI (Roche Molecular, Mannheim, Germany) as previously reported [14]. C. dubliniensis was differentiated from C. albicans by additional digests with B1n (AvrII) (Roche Molecular) [15]. In cases where the PCR-RFLP pattern did not match reported Candida patterns, further analysis using the RapID yeast plus system (Remel, Kansas, USA) and ITS region sequencing was used.

Statistical analysis

Quantitative data were analyzed using the Independent-Sample t-test. The data were compared using the Chi-Square test. All data were analyzed using the Statistical Package for Social Sciences (SPSS, Chicago, IL) version 15.0 statistical software.

Results

Of the 114 individuals with complete dentures recruited for this study, 79 (69.3%) were female. Following examination of the oral cavity, erythema was found in 53 (46.5%), and was the most prevalent clinical sign of denture stomatitis, followed by leukoplakia in 36 (31.6%), angular cheilitis in 11 (9.6%), and pseudomembrane formation (thrush) in 3 cases (2.6%). The oral cavities of 100/114 denture wearers (87.7%) were colonized with yeast. Cultures of the palatal mucosa and dentures of these subjects yielded 147 yeast isolates. Based on molecular and physiological methods, the most frequently isolated species was C. albicans with 61 isolates (41.5%), followed by C. glabrata with 27 (18.4%), C. tropicalis with 19 (12.9%), C. dubliniensis with 16 (10.9%), C. parapsilosis with 9 (6.1%), and C. krusei with five (3.4%).

We also identified three isolates of Trichosporon capitatum (2.0%), three of C. guilliermondii (1.4%), 2 of Saccharomyces cerevisiae (1.4%), and one each of C. lipolytica (0.7%) and T. beigeli (0.7%), along with one isolate suspected to be Prototheca wickerhamii (0.7%). Combinations of species isolated as mixed cultures are in Table 1. Mixed colonization with two yeast species was observed in 35 cases (31.2%), and colonization with three species was observed in 6 cases (5.4%).

Using previously established criteria [16], isolation of ≥50 Candida colonies was defined as Candida-associated denture stomatitis or infection. Culture-positive cases were divided into two groups (fewer than and greater than or equal to 50 colonies) based on colony counts. Chi-square analysis of patient demographics, including sex, smoking habits, xerostomia, denture-cleaning methods, daily frequency of use, duration of denture use, denture vertical dimensions, and denture suction was performed against Candida-associated denture stomatitis. Duration of denture use was the only factor that had a significant impact on
infection status as defined by isolation of ≥50 colony forming units (CFUs). Candida-associated denture stomatitis was identified in 1/4 (25%) of individuals using dentures for less than one year, in 8/15 (53.3%) individuals using dentures for 1–5 years, and in 75/89 (84.2%) of individuals using dentures for more than 5 years (P < 0.05). When denture users of less than 5 years were compared to those using dentures for more than 5 years, the significance was even greater (P < 0.001). No significant differences were found between the Candida species identified and infection.

Discussion

Denture-related stomatitis has a multifactorial etiology that is associated with denture use, and disease presentation is affected by both endogenous and exogenous factors [3,6]. A critical risk factor, however, is colonization of the oral mucosa by Candida species [3,9]. Previous reports demonstrated that C. albicans was responsible for about 54–74% of denture-related stomatitis cases [6,8,17,18]. However, in our study, one-fifth (20.7%) of isolates were reclassified as C. dubliniensis after BlnI restriction-enzyme digests of what was presumed to be C. albicans DNA, and the results were confirmed by sequencing [15].

The isolation of C. albicans together with C. dubliniensis from 77 patients (52.4%) in this study was similar to results in other studies that did not discriminate between these two species [6–8,17,18]. Our study is the first to characterize and report the presence of C. dubliniensis in Iran. In similar results from Gasparoto et al. [20], isolation of this species from about 10% of denture wearers showed that the prevalence of C. dubliniensis has been underestimated because of misidentification. Recent reports on denture wearers identified either no C. dubliniensis isolates, or only a single isolate, even with the use of molecular methods capable of differentiating C. albicans from C. dubliniensis [19,21]. Therefore, additional analysis of different patient populations is needed to determine the role and colonization rates of this species in the oral cavities of denture wearers.

Although C. albicans has been shown to be the predominant isolate recoverable from the oral mucosa of denture wearers, other species have also been identified [22]. Our data support this observation, similar to previous reports [7,23–25]. C. glabrata was the second most frequently isolated species in our study, although others found the second most prevalent isolate to be C. tropicalis [26]. In addition, the infrequently isolated species C. lipolectica was also isolated and identified in this study. This species was recently isolated from a denture stomatitis case in conjunction with C. albicans in a female patient, and was the sole etiologic agent in a male denture stomatitis case [7], suggesting that it can be considered a causative agent of oral candidiasis. We also report a suspected P. wickerhamii isolate that co-cultured with C. albicans from a 64-year old man with good denture hygiene and without clinical signs of denture stomatitis. This isolate had more than 99.9% similarity to P. wickerhamii based on the RapID pattern (Microcode: 730031). However, using ITS sequencing, it had 99% similarity to C. ethanolica, and 97% similarity to Picha sp. Evidence is increasing that more than one Candida species may be simultaneously recovered from one sample [6,7,27]. Using chromogenic medium and molecular methods, we identified 35 cases of mixed colonization with two species, and six cases with three species. The higher rates of mixed colonization in this study compared to previous reports [6,18] might be due to using multiple isolation techniques.

Isolation of Candida species in high numbers (≥50 CFU) defines Candida-associated denture stomatitis [16]. Based on this criterion, 77.1% (n = 88) of the cases had Candida-associated denture stomatitis, paralleling data from previous reports [8,18]. However, in contrast to work by Kurnatowska et al. [17], we found no direct association between infection (≥50 CFU) and denture stomatitis presentation. These differences may be attributed to

Table 1 Distribution frequency of mixed colonization of the yeasts isolated from the oral cavities of complete denture wearers.

<table>
<thead>
<tr>
<th>Species</th>
<th>Number (%)</th>
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<th>Number (%)</th>
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<tr>
<td>C. glabrata + C. albicans</td>
<td>6 (5.3%)</td>
<td>C. krusei + C. parapsilosis</td>
<td>2 (1.8%)</td>
</tr>
<tr>
<td>C. glabrata + C. tropicalis</td>
<td>6 (5.3%)</td>
<td>C. tropicalis + T. beigeli</td>
<td>1 (0.9%)</td>
</tr>
<tr>
<td>C. glabrata + C. dubliniensis</td>
<td>5 (4.4%)</td>
<td>C. tropicalis + C. dubliniensis</td>
<td>1 (0.9%)</td>
</tr>
<tr>
<td>C. glabrata + C. parapsilosis</td>
<td>2 (1.8%)</td>
<td>C. tropicalis + C. parapsilosis</td>
<td>2 (1.8%)</td>
</tr>
<tr>
<td>C. glabrata + C. krusei</td>
<td>1 (0.9%)</td>
<td>C. glabrata + C. dubliniensis + C. albicans</td>
<td>1 (0.9%)</td>
</tr>
<tr>
<td>C. albicans + C. dubliniensis</td>
<td>2 (1.8%)</td>
<td>C. glabrata + C. dubliniensis + C. tropicalis</td>
<td>1 (0.9%)</td>
</tr>
<tr>
<td>C. albicans + T. capitatum</td>
<td>2 (1.8%)</td>
<td>C. glabrata + C. dubliniensis + C. tropicalis + C. guilliermondii</td>
<td>1 (0.9%)</td>
</tr>
<tr>
<td>C. albicans + C. tropicalis</td>
<td>2 (1.8%)</td>
<td>C. dubliniensis + C. tropicalis + C. lipolectica</td>
<td>1 (0.9%)</td>
</tr>
<tr>
<td>C. albicans + P. wickerhamii</td>
<td>1 (0.9%)</td>
<td>C. dubliniensis + C. guilliermondii + S. cerevisiae</td>
<td>1 (0.9%)</td>
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<tr>
<td>C. albicans + S. cerevisiae</td>
<td>1 (0.9%)</td>
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<tr>
<td>C. albicans + C. krusei</td>
<td>1 (0.9%)</td>
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discrepancies in the definition of candidiasis (infection vs. colonization), or the use of different infection thresholds. Similar to previous reports [6,28], we found that candidiasis in denture wearers increased with duration of denture use. No significant correlation was found between the Candida species and Candida CFUs.

Since dentures and age-related immunosuppression are both well-known risk factors associated with candidiasis development [29], 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. Although C. albicans was the most frequently isolated species, our results also demonstrated a relatively high prevalence of C. dubliniensis in complete denture wearers, which might be misclassified as C. albicans.

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