Occurrence Rate of Oral Candida Species in Edentulous Denture Wearers Dentate Subjects

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\textbf{A B S T R A C T}

\textbf{Background and Aims:} Different Candida species, especially Candida albicans have been known as part of human oral cavity normal flora. Changes in the oral environment resulting from tooth loss or denture application can affect oral microflora. The general purpose of the current study was to determine Candida species occurrence rate in the oral cavity of denture wearer patients in comparison with those without denture.

\textbf{Materials and Methods:} A total 30 edentulous elderly with complete removable denture and 30 dentulous elderly people, who had been admitted for non-prosthetic treatments, were randomly selected in Yazd dentistry department. Their oral rinse samples were collected for mycological examination, and cultured on CHROMagar Candida plates. Frequency and density of Candida species isolated from both groups were compared using SPSS software with T-test, and differences were considered significant at \( p<0.05 \).

\textbf{Results:} Oral Candida species were isolated from 63.3\% of edentulous people with dentures in comparison with 33.3\% dentulous elderly persons (\( p=0.001 \)). Non-albicans Candida species were isolated more frequently in denture wearers compared with the dentulous group (\( p=0.03 \)). There was no significant difference between both groups in case of Candida albicans isolation (\( p=0.09 \)).

\textbf{Conclusion:} The findings of the current study show that long-term use of dentures in edentulous denture users can result in a wide Candida species colonization, causing denture stomatitis.

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Introduction

*Candida* species, particularly *Candida albicans* have been recognized as commensal human normal flora isolated from different parts of human body. There are a wide variety of microorganisms in the normal human oral cavity covering 300 to 400 species including 20 species of *Candida*. *Candida* species are present in the oral cavity of almost half of the population. Some alterations such as tooth loss, and application of the complete removable denture associated with poor oral hygiene in edentulous patients may cause their overgrowth [1, 2]. Although, oral *Candida* is not harmful in normal healthy hosts, but several predisposing factors such as old age, immunosupression, diabetes, leukemia, acquired immunodeficiency syndrome and neoplasia (particularly head or neck cancer) may cause opportunistic candidiasis infection. Oral candidiasis has been reported to be associated with candidiasis in the lung and also deglutition pneumonia [3]. Literature studies revealed a causal relationship between denture wearing and persistent or even recurrent denture stomatitis as a result of the initial adhesion of *Candida* species to the denture base materials such as acrylic resin and polymethyl methacrylate [4, 5].

Denture stomatitis is usually a chronic inflammatory disorder of palatal and alveolar mucosa covered with removable dental prostheses, and is associated with burning, bleeding, an unpleasant taste, or halitosis in complete removable denture users [6]. However, the prevalence of denture stomatitis is ranged from 15 to 65% in edentulous community, while in institutionalized denture wearing population it may reach at up to 72% [7, 8]. However, multi-factorial etiology for this mucosal infection has long been reported, including trauma caused by ill-fitting dentures, microbial biofilm, poor denture hygiene, continuous denture wearing habits and several other systemic factors. Numerous studies highlighted the main role of *Candida albicans* in the development of denture stomatitis [1, 9, 10].

The aim of this analytical-descriptive study was to compare the frequency and density of different *Candida* species isolated from oral cavity of edentulous denture wearer and elderly subjects wearing their own dentition.

Materials and Methods

In the current cross-sectional study, 30 edentulous elderly people having complete removable denture and 30 edentulous patients, who referred to Yazd dentistry department for non prosthetic treatments, were randomly selected. In order to reach minimum 5 unit differences in the average microbial colony counts between the groups, the significant level of 5%, \(\alpha=5\%\), \(\beta=2\%\), \(S=80\%\) using the formula, 30 subjects were opted for each group. The elderly subjects with the history of underlying diseases such as malignancies, diabetes, neoplasm, immunosuppression, and current antibiotic users were excluded from the
present study. All subjects were in the same age range and matched based on sex. All subjects completed an informed consent form to participate before being accepted for oral sample collection.

**Sample collection and detection of Candida species**

Only subjects passing more than two hours from their last eating and brushing were included in the present study. The patients were requested to rinse their mouth with 10 ml of sterile phosphate-buffered saline (PBS, 0.1 M, pH 7.2) for one minute (denture had to be removed prior to sampling in denture users) and then to return the oral rinse to a sterile universal container. All oral rinse samples were concentrated by centrifugation (at 1700 g for 10 min), and the supernatant was discarded. The deposit was re-suspended in 1 ml of sterile PBS, and a 10 µl sample was inoculated on the CHROMagar Candida plates (CHROMagar, France) by the method of Beighton et al. [12], and incubated for 48 to 72 hrs at 37ºC. The plates were then assessed for identification and enumeration of isolated Candida species based on the color of Candida colonies and the media chart. The colony forming unit (CFU) was multiplied by the dilution factor to yield the CFU per milliliter of original oral rinse sample for each individual [13].

The study was approved by the Ethics Committee of Shahid Sadoughi University of Medical Sciences.

**Statistical Analysis**

Frequency and density of recovered Candida Spp. colonies (CFU/ml) isolated from both groups were compared using SPSS software with T test, and differences were considered significant at p<0.05.

**Results**

Positive oral rinse samples with Candida species were more frequently seen in edentulous denture wearers compared with dentulous subjects (Table 1).

<table>
<thead>
<tr>
<th>Variables</th>
<th>Edentulous denture wearer</th>
<th>Elderly edentulous subjects</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>18 male, 12 female</td>
<td>15 male, 15 female</td>
<td>0.341</td>
</tr>
<tr>
<td>Age</td>
<td>57.5±12</td>
<td>53.5±15.5</td>
<td>0.106</td>
</tr>
<tr>
<td>Candida positive oral sample</td>
<td>19</td>
<td>10</td>
<td>0.001</td>
</tr>
<tr>
<td>Positive samples with C. albicans</td>
<td>9</td>
<td>6</td>
<td>0.08</td>
</tr>
<tr>
<td>Non-albicans positive samples</td>
<td>10</td>
<td>4</td>
<td>0.03</td>
</tr>
</tbody>
</table>

**Table 1.** Subject's variables and the frequency of positive Candida samples in edentulous denture wearers and edentulous subjects
There was a statistically different higher positive culture in denture users in comparison with subjects without denture (p=0.001). However there was no statistically significant difference between the frequency of isolated samples with *Candida albicans* (p=0.12) between both groups, but non-albicans *Candida* species were mostly seen (Fig. 1) In edentulous denture users compared with the elderly people with normal dentition (p=0.03). In case of *Candida* density in oral samples, a higher density of *Candida* spp. colonies was recovered from edentulous denture users in comparison with edentulous elderly persons except for *C. glabrata* (Table 2).

**Fig. 1.** Isolation frequencies of oral *Candida* species in edentulous denture wearers and dentate subjects

**Table 2.** Density (Mean±SD, CFU/ml) of recovered *Candida* species in edentulous denture wearers and dentulous subjects

<table>
<thead>
<tr>
<th><em>Candida</em> species</th>
<th>Edentulous denture wearer</th>
<th>Elderly edentulous subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. albicans</em></td>
<td>57.5±18.3</td>
<td>44.6±11.9</td>
</tr>
<tr>
<td><em>C. tropicalis</em></td>
<td>32.4±12.7</td>
<td>27.2±11.3</td>
</tr>
<tr>
<td><em>Candida krusei</em></td>
<td>18±8.3</td>
<td>19.8±9.1</td>
</tr>
<tr>
<td><em>C. kefyr</em></td>
<td>24.5±7.8</td>
<td>0</td>
</tr>
<tr>
<td><em>C. glabrata</em></td>
<td>0</td>
<td>13.2±2.8</td>
</tr>
</tbody>
</table>
Discussion

In Iran, the size of elderly population has increased, and this requires corresponding improvements in their health and medical care. Citizens who were older than 65 years of age represented 3.1% of the overall Iranian population in 1986, 5.1% in 2006, and are expected to reach 10% in 2035 [14]. Prevention of elderly oral diseases and improving their oral health care is a prerequisite for better quality of life in this population. Preserving of 20 teeth or more is ideally needed to warrant an acceptable mastication and food intake even up to the age of 80 years [15].

Candida species are reported as the most frequent opportunistic fungi, causing candidiasis in different areas of human body including oral cavity. Since these fungi are commensally presented in human oral cavity, their colonization can promote oral candidiasis, with its different manifestations [16]. Denture stomatitis, as a mucosal manifestations of oral candidiasis in elderly denture users, results from attachment and colonization of Candida species on denture hard surface.

The present study was conducted in order to show the role of denture on the frequency and density of oral Candida colonization in denture users and normal edentulous subjects [17]. Aging has been reported as the cause of progressively increasing incidence of Candida in the human oral cavity. However, in the current study, an increase in positive oral Candida particularly non-albicans Candida species was seen in edentulous elderly denture wearers in comparison with dentate people [15]. The results of the present study showed a higher frequency of positive Candida species in oral rinse specimens of edentulous denture than samples of those without denture. Oral Candida species were isolated from 63.3% of edentulous people with dentures, a finding supported by Budetz-jorgencen [18] study, who isolated them in 65% of denture users. Our results differ greatly from 85% found by Arirachakaran study [10]. The differences may be explained by different diagnostic criteria or differences in the groups including age, institutionalized versus non-institutionalized subjects, or even the influences of other factors such as diseases or drugs.

Candida albicans was the most commonly isolated species isolated from oral rinse culture in the present study, which is similar to that HE’s et al. study [19]. However, a statistically significant higher frequency and density of non-albicans Candida species also were recovered from oral rinse in denture wearer samples in comparison with those without denture. Recently a shift from Candida albicans towards non-albicans species recovered from oral candidiasis and denture stomatitis lesions has been observed [20, 21]. Unfortunately, these species are more resistant to common antifungal drugs than C. albicans. This further attention to oral and denture hygiene of denture users in order to control possible oral candidiasis and involvement of adjacent organs like lung and gastrointestinal tissues in asthenia immunosuppressed elderly.
A high prevalence of colonization of *Candida* spp. is often documented in elderly denture wearers [6, 23]. *Candida* species adhere to acrylic surfaces of dentures which plays an important role for the colonization and in turn in the pathogenesis of oral candidiasis [6, 24].

**Conclusion**

Findings from the present study indicated more frequent and density of *Candida species* especially non-albicans *Candida species* in patients with dentures in comparison with non-denture wearers highlighting the role of denture in denture stomatitis and the need for better oral as well as denture hygiene in edentulous denture wearers.

**Conflict of Interest**

The authors declare that they have no conflicts of interest.

**Acknowledgement**

This article resulted from a part of dentistry student thesis, and was financially supported by Vice Chancellor for Research and technology of Shahid Sadoughi University of Medical Sciences. The authors would like to thank Ms Mahin Ghafoorzadah for her kind help in the laboratory works.

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