Is denture stomatitis always related with candida infection?  
A case control study

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ABSTRACT

Aim To examine the oral mucosa under the denture base and to determine the influence of local factors that contribute to denture stomatitis.

Methods In this prospective, case control study 30 patients with palatal inflammatory lesions were evaluated. A degree of palatal inflammation was scored. Swab samples were taken from tongue and palatal mucosa for microbiological examination. Denture plaque index, data of night wearing dentures, pH values of tongue and palatal mucosa were determined for all subjects.

Results Significantly higher incidence of poor denture cleanliness index (p=0.01) and night wearing of dentures (p=0.009) were found in patients with denture stomatitis. There were significant differences between the groups in relation to the pH value of the tongue and palatal mucosa (p=0.016 and p=0.035, respectively). No significant association was found between denture stomatitis and microbiological findings, dentures age, type of dentures, presence of previous prosthesis, frequency or manner of dentures hygiene and smoking habits.

Conclusion Poor denture hygiene, overnight wearing of dentures and oral mucosa pH less than 6.5 are predominant local etiological factors that contribute to denture stomatitis development.

Keywords: dental prostheses, hygiene, oral candidiasis, etiology, dental care for elderly

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INTRODUCTION

Denture stomatitis (DS) is a clinical diagnosis of the oral mucosa inflammatory lesion, which is located under the base of removable denture (1). According to the classification proposed by Newton (2), there are three clinical types of the disease: pinpoint hyperemia (type 1), diffuse mucosal erythema, which is limited by denture base (type 2) and inflammatory papillary hyperplasia (type 3). The distribution of these lesions in removable denture wearers tends to increase with age (3). Several studies have revealed that DS is more prevalent in the female population (4,5). The etiology of DS is multifactorial, with predominance of local factors. The colonization of Candida genus yeasts, poor denture hygiene, continual and nighttime wearing of removable dentures, denture age, trauma caused by dentures and smoking are described in literature as important factors that lead to DS (1, 4, 6-9). Some authors reported the significant impact of poor hygiene in the development of inflammatory lesions on the oral mucosa (7,10), which is explained by the pathogenic influence of microorganisms (Candida spp., Staphylococcus spp., Streptococcus spp. and Enterobacteriaceae/Pseudomonadaceae families) being part of the denture plaque (11,12). The association of Candida albicans colonization and palatal mucosa lesions has been mostly described in the previous studies (5,7,10,12). According to Budtz-Jorgensen (9), invasion of yeast into the tissues, production of virulence factors and induction of hypersensitivity reactions have been described as possible mechanisms in initiation of the disease. The impact of overnight denture wearing is also cited as an important predictor in the pathogenesis of denture stomatitis (1,13). In continuous wearing, a decline in the pH value of palatal mucosa due to acid products of yeasts, lactobacilli and streptococci is noted. Reduced salivation at night also contributes to acid environment between the denture base and oral mucosa (9). However, Yilmaz et al. (14) found no significant association between night-wear and denture stomatitis. There is no control study that observed all previously reported etiology factors at the same time and there is a lack of information about oral mucosa pH value and its relation with Candida infection under the base of removable denture.

The aim of this study was to examine the oral mucosa which is in direct contact with the base of the prosthesis and to determine the presence and influence of local factors (purity of dentures, denture age, night wearing of dentures, palate and tongue mucosal pH and Candida infection on the oral mucosa) in the development of denture stomatitis in upper removable dentures wearers.

PATIENTS AND METHODS

The study was conducted as a prospective, case control clinical study at the Oral Medicine Section of the Dental Clinic, School of Medicine, Novi Sad, Serbia between January 2012 and August 2013. The study group involved 30 consecutive patients with upper removable dentures that had inflammatory lesions on the mucosa of the alveolar ridge and the hard palate (21 female and 9 male subjects of average age of 66.30 years (ranged 60-87 years). The control group consisted of 30 healthy subjects with upper dentures and no oral mucosa lesions (25 females and 5 males of average age of 65.30 years (ranged 58-76 years). Subjects with upper removable dentures (complete dentures, acrylic partial dentures, cast partial dentures) older than 2 years were included in the study. Criteria for exclusion from the study were: the presence of systemic disease (immune, neoplastic, infectious, and endocrine diseases), antibiotic or corticosteroid therapy within the previous 6 months and the presence of a dry mouth sense (xerostomia).

All subjects were interviewed using a structured questionnaire by one investigator in order to minimize possible variations in the data gathering. Visual examination of the mouth was carried out by a specialist in oral medicine experienced in that field. Information explaining the content of the study and purpose of the research results, was provided to patients in writing. Having learned about the procedure, the patients gave their written consents. The study was approved by the Ethical Committee of School of Medicine in Novi Sad.

A questionnaire that included questions with anamnestic predetermined sequence: generalities, socio-demographic information, gender, age, height and weight, data of harmful habits (smoking and alcohol consumption), the frequency of visiting the dentist, information about prosthetic restoration (prosthesis type, length of time wearing dentures, presence of previous prosthesis, information about the night wearing dentures, frequency and manner of dentures hygiene), frequency and method of ma-
taining dental hygiene, was completed for each subject. During the clinical examination, condition of oral mucosa and dental status were established. Based on hard palate and alveolar ridge mucosa clinical examination in the patients with inflammation, the degree of palatal inflammation was scored: 0-no inflammation; 1-slight inflammation (localized slight hyperemia); 2-moderate inflammation (diffuse hyperemia); 3-severe inflammation (diffuse and papillary hyperplasia) (15).

Denture plaque index was measured for each prosthesis. Denture base that is in direct contact with oral mucosa was coated with 1% solution of gentian violet and then dentures were washed under running water. Denture plaque index was determined according to Budtz-Jorgensen: excellent (absence or presence of plaque in the form of a few points), satisfactory (less than half of the surface of denture base covered with plaque) and bad (more than half of the surface of denture base covered with plaque) (16).

Palatal mucosa and tongue pH values were determined using standard pH paper indicator with a sensitivity of 0.5 (Neutralit, Merck, Darmstadt, Germany). The pH values from 6.5-7.0 were considered normal (17). The pH paper was placed and gently pressed on the hard palate and dorsal surface of the tongue for 5 s, and immediately after that the mucosa pH values were determined comparing the color change of the paper with the attached scale. According to the measured pH value of palatal and tongue mucosa, participants were classified into two groups: group with acid pH value (pH<6.5) and group with normal and alkaline pH value (pH≥6.5).

Swab samples from tongue and palate (each lasting 10-15 seconds) for mycological and bacteriological examination were taken from the both groups. All samples were taken in the morning (from 9 to 11 am), and reached the laboratory within 2 hours. All swab samples were taken by one investigator. Swab samples were plated on Sabouraud dextrose agar (Difco, Detroit, MI, USA) and incubated under aerobic conditions at 37 °C for 48 h. Inoculated culture media was observed after 48 h in order to detect the presence of clinically significant species of bacteria and yeast in the examined material.

RESULTS
Comparing the average age and gender of the patients wearing denture there was no significant difference between the groups (p>0.05). According to degree of the hard palate mucosal inflammation, it was found that 10 (33.3%) patients with DS had slight, while 20 (66.7%) had moderately or severely inflamed palatal mucosa (p<0.05).

<table>
<thead>
<tr>
<th>No (%) of patients</th>
<th>Denture plaque index</th>
<th>Control group n=30</th>
</tr>
</thead>
<tbody>
<tr>
<td>Excellent</td>
<td>2 (6.7)</td>
<td>11 (36.7)</td>
</tr>
<tr>
<td>Bad</td>
<td>28 (93.3)</td>
<td>19 (63.3)</td>
</tr>
</tbody>
</table>

| Table 1. Denture cleanliness index data in the groups |

The bad denture cleanliness index was detected in 28 (93.3%) patients in the DS group and 19 (63.3%) patients from the control group. There was significant difference in denture cleanliness index between the groups (p=0.01). Due to the small number of patients with satisfactory index and for the purpose of an adequate statistical analysis, subjects with satisfactory and bad index were merged into one group (bad) (Table 1).

The statistical analysis revealed a significantly higher prevalence of night wearing dentures in patients with DS, 73.3% (22) than in the control group, 36.7% (11) (p=0.009) (Table 2).

<table>
<thead>
<tr>
<th>No (%) of patients</th>
<th>Night wearing dentures</th>
<th>Control group n=30</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>22 (73.3)</td>
<td>11 (36.7)</td>
</tr>
<tr>
<td>No</td>
<td>8 (26.7)</td>
<td>19 (63.3)</td>
</tr>
</tbody>
</table>

| Table 2. Prevalence of night wearing dentures in the groups |

Acid pH value of palatal mucosa under the base of removable denture (pH<6.5) were found in 20 (66.6%) patients with DS, and 10 (33.3%) patients from the control group (Table 3). There were significant differences between the groups in relation to the pH value of the palate (p=0.016).
Table 3. pH value of palate and tongue mucosa in the groups

<table>
<thead>
<tr>
<th>Mucosa</th>
<th>No (%) of patients</th>
<th>n=30</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Palate</td>
<td>Tongue</td>
<td>Palate</td>
</tr>
<tr>
<td>pH&lt;6.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>1 (3.3)</td>
<td>2 (6.7)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>5.5</td>
<td>0 (0)</td>
<td>1 (3.3)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>6</td>
<td>19 (63.3)</td>
<td>19 (63.3)</td>
<td>10 (33.3)</td>
</tr>
<tr>
<td>pH&lt;6.5</td>
<td>10 (33.3)</td>
<td>8 (26.7)</td>
<td>20 (66.6)</td>
</tr>
<tr>
<td>7</td>
<td>9 (30.0)</td>
<td>5 (16.7)</td>
<td>19 (63.3)</td>
</tr>
<tr>
<td>8</td>
<td>1 (3.3)</td>
<td>3 (10.0)</td>
<td>1 (3.3)</td>
</tr>
</tbody>
</table>

The pH<6.5 on the dorsal surface of the tongue was measured in 22 (73.4%) subjects with DS and 12 (40%) control subjects. Significant differences were also found between the groups in relation to the pH value of tongue (p = 0.035).

Of the total 20 patients with pH<6.5 on palatal mucosa, Candida species were isolated in 9 (45%) patients with DS. Positive microbiological finding from the palate and the tongue was confirmed in 13 (43.3%) and 19 (63.3%) subjects with denture stomatitis respectively, while in the control group 11 (36.7%) subjects had positive microbiological finding from the tongue or the palate mucosa (p = 0.120 and p=0.596, respectively). In DS group, Candida albicans, Candida spp. and bacteria were isolated from the hard palate mucosa in eight (26.7%), five (16.7%) and two (6.7%) patients respectively, while in the control group both Candida species were found in four (13.3%) and bacteria in two (6.7%) patients. Candida albicans from the dorsal surface of the tongue was isolated in 10 (33.3%) patients with DS and five (16.7%) from the control group, while Candida spp. was present in nine (30%) patients with DS and six (20%) control subjects. Bacteria (Pseudomonas aeruginosa and Escherichia coli) were isolated in four (13.3%) patients with denture stomatitis and one (3.3%) control patients.

There were no significant differences between the groups in relation to dentures age (p=0.667), type of dentures (p=0.954), presence of previous prosthesis (p=1.000), frequency or manner of dentures hygiene (p=0.362) and cigarette smoking habits (p=0.754).

**DISCUSSION**

In the present study the condition of palatal mucosa and influence of several local etiological factors on the occurrence of the disease have been analyzed. Our results confirmed DS multifactorial etiology. Poor denture hygiene was significantly more present in patients with palatal inflammatory lesions compared to the control group. Significant correlation that was found between poor index of denture cleanliness and DS has been reported in several previous studies (4,6-8, 14). The current findings show high prevalence of subjects with bad denture cleanliness index and this result falls in the range reported previously (7,10). Although Naik et al. (10) did not find significant difference between DS and healthy subjects in relation to denture cleanliness, their results show that poor denture hygiene is directly proportional with the intensity of palatal inflammation. Kossioni (18) also points out the association between poor dentures hygiene and inflammatory changes of palatal mucosa in the form of papillary hyperplasia. Also, the possible cause of poor dentures hygiene could be a lack or inadequate instructions from the dentist or dental hygienist with regard to the method and technique of hygiene.

The overnight or continuously denture wearing was present in 73.3% of subjects with palatal inflammation. The impact of night wearing of dentures on the occurrence of the disease was supported by Jagana-than et al. (19), in which study overnight wearing was present in 61% of subjects with palatal diffuse erythema. Similar results were reported by Kossioni (18), who found that 56% of respondents wore dentures at night. However, some authors did not confirm the influence of this habit in DS patients (14). It is important that production of saliva is reduced at night (9). Therefore, the presence of denture further hinders its antimicrobial role. The absence of mechanical effects of saliva and its antimicrobial compounds that are an integral part of saliva (lysozyme, lactoferrin, salivary peroxidase, immunoglobulin A) during the night as possible mechanisms which contributes to inflammation, should indicate a need to be explored in further studies.

The current study showed that the oral mucosa pH values <6.5 were over-represented in patients with palatal lesions (66.6%) compared to the control group (33.7%). Comparing the pH values of denture biofilms, Olsen et al. (20) have reported the differences between the groups with and without palatal inflammation. Same authors suggest that the difference in pH values between the groups was not accompanied by differences in positive microbiological findings (genus Candida yeasts and bacteria) of palate and tongue, and they did not find a significant increase in the number of yeasts in conditions of pH decrease (21). It should be noted that the pH values in those subjects were measured on denture biofilm.
using microelectrodes, and yeasts were isolated from denture biofilm. While in the present study, which suggests similar results, pH values of the oral mucosa from which swabs were taken for microbiological examination were measured.

The association between DS and yeast colonization has been reported frequently by most of authors (5,7,10,12,22). In DS group, we found more positive microbiological findings on the palate and tongue with no significant differences compared with the control. Positive swab results of microbiological analysis from the oral mucosa accompanied by clinical signs and symptoms seen for diagnosis of oral candidiasis rather than determining the colonization of the oral cavity (23), which is previously reported as the most frequent factor related with DS (5,7,10,12). The presence of *Candida* on the tongue and palate in denture wearers with inflammatory oral lesions may indicate the existence of previous oral carriage of *Candida* species. Symptom-free oral carriage of *Candida* species with the prevalence in clinically normal mouths of healthy adults from 3 to 48% has been reported (7,24), with significant increase in proportions in those wearing dentures (25). In this study, 36.7% of control subjects had positive mycological findings (genus yeast) on tongue or palate. These data suggest the reason why we did not obtain the significant differences between the groups in relation to mycological findings of the oral mucosa. Similar conclusion that mucosa candidal infections are not the predisposing factor in the occurrence of denture-induced stomatitis, but they play a major role, has been reported in two recent studies (15,26). The decline of the pH values may also contribute to yeasts reproduction and development of a clinically visible *Candida* infection on the palate. There are many suggested factors except dentures and decline in pH value that can influence *Candida* infection. Depression in normal host defense is undoubtedly a major factor that can predispose an individual to yeast colonization, promote yeast overgrowth and established candidiasis (27). According to the review of the literature on the epidemiology and etiology of denture stomatitis, Gendreau and Loewy (28) suggest that etiological factors such as poor denture hygiene, continual and nighttime wearing of removable dentures, accumulation of denture plaque and poor-fitting dentures appear to increase the ability of *Candida albicans* to colonize both the denture and oral mucosal surfaces, where it acts as an opportunistic pathogen. Moreover, except *Candida* species in some dentured patients, aerobic gram-negative bacteria (*Pseudomonas aeruginosa* and *Escherichia coli*) have been isolated from the tongue or palate. Co-aggregation between *Candida* species and several other microorganisms (*Staphylococcus* spp. and *Enterobacteriaceae/Pseudomonas* families) may play important roles in the establishment and persistence of DS in complete denture wearers (11,12). Similar opportunistic and multiresistant bacteria, that are considered to belong to the transient oral microflora, we found in our previous work on healthy men smokers with poor oral hygiene (29). Colonization of these bacteria increases with age, poor oral hygiene, smoking, nail-biting or close contact with animals (30). Thus, elderly patients should be suggested that before handling removable dentures they need to thoroughly wash their hands.

According to the results obtained, this study suggests that poor denture hygiene, overnight wearing of dentures and oral mucosal pH less than 6.5 are the predominant local factors that contribute to denture stomatitis development. Patient education and motivation about denture hygiene and their removing at night are the most important steps in the prevention of the oral mucosal diseases in dentate patients.

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No specific funding was received for this study.

**TRANSPARENCY DECLARATION**

Competing interests: None to declare.

4. Santos CM, Hilgert JB, Padilha DMP, Hugo FN. Denture stomatitis and its risk indicators in south Brazil-

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SAŽETAK

Cilj Ispitati stanje oralne sluzokože koja je u direktnom kontaktu s bazom proteze, te utvrditi prisustvo i uticaj lokalnih faktora u nastanku protetskog stomatitisa.

Metode Istraživanje je sprovedeno kao prospektivna studija s kontrolnom grupom kod 30 pacijenata s gornjim mobilnim protezama, koji su imali inflamatorne promene na sluzokoži alveolarnog grebena.

Rezultati Kod ispitanika s protetskog stomatitisom značajno je veća učestalost loše higijene proteze (p=0,01) i noćnog nošenja proteze (p=0,009). Utvrđena je značajna razlika u vrednostima pH sluzokože jezika i nepca između grupa (p=0,016 odnosno p=0,035). Značajna povezanost nije utvrđena između protetskog stomatitisa i mikrobiološkog nalaza, starosti proteze, vrste protetske nadoknade, prisustva ranjih proteza, učestalosti održavanja higijene proteze i pušenja cigareta.

Zaključak Lođa higijena proteza, noćno nošenje i pH vrednost oralne sluzokože, manji od 6,5 predstavljaju najznačajnije lokalne etiološke faktore u nastanku protetskog stomatitisa.

Ključne reči: zubna proteza, higijena, oralna kandidijaza, etiologija, stomatološka nega starih

Da li je protetski stomatitis uvek povezan s infekcijom kandidom

– studija “slučaj-kontrola”
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