



## Microbial Colonization and their Relation with Potential Cofactors in Patients with Denture Stomatitis

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### ABSTRACT

**Aim:** To determine microbial prevalence in the mucous membrane and prosthesis of denture wearers with and without denture stomatitis and to study its relationship with potential clinical cofactors.

**Materials and methods:** Saliva was collected from 200 patients (100 female and 100 male) wearing dental prosthesis for measurement of pH. Oral samples of the mucous membrane and of dentures were taken with sterile swab for microbiological analysis. Medical and dietary history of the subjects was recorded. Chi-square test and analysis of variance were used for statistical analysis.

**Results:** Diabetes, hypertension and high carbohydrate ingestion was observed in majority of patients. The pH average in saliva was of 5.1. The presence of *C. albicans*, *S. aureus* and *S. mutans* in the mucous membrane was of 55.4, 56.4 and 63.6% respectively. *C. albicans* was isolated in 69.7% from the prosthesis, whereas *S. aureus* and *S. mutans* were isolated in 45.3%. The isolation of *C. albicans* was more frequent in patients with denture stomatitis.

**Conclusion:** *C. albicans*, *S. aureus* and *S. mutans* frequently colonize the oral mucous of denture wearers. This is more frequently observed in patients with denture stomatitis.

**Clinical significance:** Denture stomatitis is associated to *Candida albicans*, different bacteria and other cofactors, such as salivary pH, carbohydrate ingestion, systemic illnesses and medication.

**Keywords:** Denture stomatitis, *Candida albicans*, Carbohydrate, Salivary pH.

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**Conflict of interest:** None declared

### INTRODUCTION

Denture stomatitis is the term used for the chronic inflammatory changes of the denture bearing mucosa, which

is characterized by erythema of the palate and the alveolar ridges.<sup>1</sup> Strains of the genus *Candida* are very often involved as a causative factor in denture stomatitis. Still, this condition is not a specific disease entity as other causal factors exist, such as bacterial infection or mechanical irritation. Mechanical irritation may possibly play a predisposing role by increasing the turnover of the epithelial cells, hence, the barrier function of the epithelium is reduced and its penetration by microbial antigens is possibly enhanced.<sup>2-4</sup>

Risk factors associated with denture stomatitis are wearing complete (in contrast to partial) dentures, maxillary (in contrast to a mandibular) removable denture, inadequate denture hygiene, denture wear at night, poor denture quality, diabetes mellitus, antibiotic therapy, immune deficiencies, vitamin A, foliate and iron deficiencies; impaired salivary gland function, xerogenic medication, tobacco use, salivary secretion rate and gender.<sup>1,5-11</sup>

*C. albicans* and *Staphylococcus aureus* are associated in lesions of several patients with queilitis angular, where *C. albicans* plays an important etiological role. *C. albicans* and *Staphylococcus aureus* are microorganisms with an elevated adhesion capacity to the oral mucous. This adherence is enhanced *in vitro* when *Candida* is incubated simultaneously with *S. mutans*, *S. sanguis*, *S. salivarius* or some other bacteria. The development of stomatitis includes important associated factors, such as changes in saliva, as well as other oral alterations.<sup>12</sup>

### OBJECTIVES

1. To determine microbial prevalence in the mucous membrane of denture wearers with and without denture stomatitis.
2. To determine microbial prevalence in the prosthesis of denture wearers with and without denture stomatitis.
3. To study its relationship with potential clinical cofactors.

## MATERIALS AND METHODS

Two hundred patients wearing complete dental prosthesis were included in the study. The subjects were recruited from the Outpatient department (OPD) of the institution. Ethical clearance was obtained from the institutional review board. Informed consent was obtained from all the subjects. The patients were divided into two groups: With denture stomatitis or without it.

### Samples Collection

The patients were asked not to eat or drink and to abstain from oral hygiene procedures before taking the samples. Patients on antimicrobial treatment in a period of at least 6 months before the study were excluded. The patients were asked to fill-up a questionnaire and were subjected to clinical check-up. A sample of 2 ml of unstimulated saliva was taken from each patient for 5 minutes. A sample of the internal surface of the prosthesis was also taken using a sterile cotton swab immediately after the prosthesis was removed from the oral cavity. A second sample was obtained from the oral mucous with atrophic denture stomatitis lesions, the palate mucous, the rear zone of the molars or any other zone in contact with the dental prosthesis using a sterile cotton swab. The samples were immediately placed in a transport media for its subsequent processing.

### Microbiological Procedures

The salivary pH was tested immediately after the sample was taken using a digital pH meter. For the isolation and identification of *C. albicans*, the samples were sown using the exhaustion technique in plates of chromogenic agar media, CandiSelect, which is a specific culture media for the growth of yeasts. It contains a nutritious base with glucose, chromogenic substrate and antibiotics in order to prevent bacterial development. Blue colonies in this media were identified as *C. albicans*. For the isolation and identification of *S. aureus*, the samples were sown in plates containing Chapman agar or mannitol salt agar, which is a selective media for pathogenic *Staphylococcus* isolation. It contains 7.5% sodium chloride and mannitol with red phenol as an acid-base marker. *S. aureus* was identified in this media by the production of golden color zones around the colonies. The samples were sown in mitis salivarius agar with bacitracin for *S. mutans* identification. The colonies of *S. mutans* appeared convex, wavy and opaque, in a dark blue color. All the plates were incubated at 37°C and evaluated for microbial growth at 24 and 48 hours. Microorganisms isolation were identified and confirmed in pure cultivation. In order to complement the identification, gram stain test and microscope observation were performed.<sup>12-14</sup>

## Statistical Analysis

All the variables were analyzed using SPSS version 16.0. *C. albicans*, *S. aureus* and *S. mutans* prevalence and its association with gender, carbohydrates ingestion and denture stomatitis was identified using the Chi-square test.

## RESULTS

Table 1 shows general clinical characteristics of the patients. One hundred and thirty-seven patients showed oral mucosa colonization by *C. albicans*. Eighty-six patients showed atrophic denture stomatitis associated with *C. albicans* presence in oral mucosa. ( $\chi^2 = 28.386$ ,  $p < 0.001$ ). *S. aureus* was isolated from the oral mucosa of 137 patients in total and from the oral mucosa in 84 patients with denture stomatitis ( $\chi^2 = 22.269$ ,  $p < 0.001$ ). *S. mutans* was isolated from 88 patients oral mucosa. Oral mucosa from only 20 patients with denture stomatitis was colonized by *S. mutans*.

Low salivary pH (5.1) as well as high carbohydrates ingestion was seen in patients with denture stomatitis in comparison with the remainder patients. It is important to point out that when the presence of denture stomatitis and the microbial colonization in mucous and prosthesis were related to other clinic cofactors. The higher percentage of systemic illnesses was hypertension ( $n = 48$ ) followed by diabetes mellitus ( $n = 64$ ). These showed a statistical correlation with denture stomatitis presence ( $\chi^2 = 30.793$ ,  $\chi^2 = 18.876$ ,  $p < 0.001$ ). No significant gender variation was seen in the present study.

**Table 1:** General clinical characteristics of the patients

Characteristics	Patients	
	Without denture stomatitis (n = 100) (%)	With denture stomatitis (n = 100) (%)
Gender (male/female)	50/50	50/50
Age (average)	65.82	64.08
Systemic illnesses		
Diabetes mellitus	19	48
Arterial hypertension	25	64
Carbohydrates ingestion		
High	68	80
Low	32	20
pH saliva (average)	5.71	5.12
Mucosal colonization		
<i>Candida albicans</i>	51	86
<i>Staphylococcus aureus</i>	53	84
<i>Streptococcus mutans</i>	68	20
Prosthesis colonization		
<i>Candida albicans</i>	68	28
<i>Staphylococcus aureus</i>	52	40
<i>Streptococcus mutans</i>	52	43

## DISCUSSION

Atrophic denture stomatitis is a frequent oral lesion in those patients wearing dental prosthesis. In this study, atrophic denture stomatitis was observed in patients with mean age of 64 years. This finding is consistent with observation in various studies that stomatitis affects mainly the elderly.<sup>5,8,12,15,16</sup> The elderly generally suffer from systemic illnesses and changes in nourishment. They also experience changes in their salivary characteristics. Also the insertion of dental prosthesis leads to alteration in the oral environment.<sup>7,8,11,15,16</sup> This was also seen in the present study, that from the 100 patients with denture stomatitis 48 were suffering from diabetes and 64 from hypertension. Pharmacological treatments change oral microflora and can change in salivary variables, hence, atrophic denture stomatitis is common patients with systemic illnesses.<sup>10,12,17</sup> The pH low conditions in saliva enhance *C. albicans* colonization.<sup>15</sup> In this study, it was seen that the salivary pH was lower in patients with stomatitis colonized by *C. albicans*, *S. aureus* and *S. mutans*, which suggests that an acid salivary pH helps denture stomatitis development.<sup>12</sup> The acid pH is also caused by a high carbohydrates ingestion diet and increases the colonization by aciduric bacteria and enhances candidial colonization. This acid ambient is found in the dorsum of tongue, in the mucous under the dental prosthesis as well as in candidosis lesions.<sup>18</sup> Samaranayake and McFarlane<sup>18</sup> have demonstrated that carbohydrates in the diet help the biofilm formation of *C. albicans* on acrylic materials. *C. albicans* was isolated more frequently from the prosthetic surface in this study. The prevailed colonization of *Candida* in the oral mucous and the prosthetic surface was bigger in this study than those observed by other authors.<sup>12,18</sup> *S. aureus* was present on the oral mucous and dental prosthesis in many patients in the present study. This finding is similar to studies by other authors.<sup>12,17</sup> It has to be considered that dental prosthesis are covered with microbial biofilms which may lead to infections though the microbial composition may vary with the type of prosthetic material.<sup>19</sup>

The treatment of denture stomatitis is principally based in disinfection, adjustment or change of dental prosthesis, use of mouth rinses and in most of the cases the administration of antifungal medication. As seen in the present study, due to the multifactor etiology and associated cofactors, therapeutic management of denture stomatitis becomes complicated. Treatment of denture stomatitis must be considered for the entire microbiota associated with it as well as the potential cofactors.

## CONCLUSION

*C. albicans*, *S. aureus* and *S. mutans* frequently colonize the oral mucous of denture wearers. This is more frequently

observed in patients with denture stomatitis. The potential cofactors include systemic illnesses, salivary pH and carbohydrate intake.

## CLINICAL SIGNIFICANCE

Denture stomatitis is associated to *C. albicans*, different bacteria and other cofactors such as salivary pH, carbohydrate ingestion, systemic illnesses and medication. Hence, the treatment of denture stomatitis should be multidimensional and not rely solely on antifungal therapy.

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