

Frequency and enzymatic activity (proteinase and phospholipase) of *Candida albicans* from edentulous patients, with and without denture stomatitis

Freqüência e atividade enzimática (proteinase e fosfolipase) de Candida albicans de pacientes desdentados totais, com e sem estomatite protética

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The so called erythematous stomatitis is frequently observed in denture wearers, being local factors, mainly related to the presence of yeasts, considered important for its development. Having these aspects in mind, we evaluated edentulous patients with and without denture stomatitis (DS), identifying the yeasts obtained from the palate, and determining the relative level of the proteinase and phospholipase exo-enzymes produced by *C. albicans*. The results suggested that *C. albicans* was the most frequent species observed, being more prevalent in patients presenting DS, isolated or in association with other yeasts, with high expression of proteinase.

UNITERMS: *Candida albicans*; Denture stomatitis; Proteinase; Phospholipase.

INTRODUCTION

Denture stomatitis (DS) comprises superficial tissue alterations, mainly under the base of upper prostheses, representing inflammatory reactions of the mucosae³. These lesions are commonly associated with the presence of complete dentures, presenting diffuse or punctuated erythematous areas, besides other unspecific aspects mainly observed on the palate. Soft tissue alterations are sometimes associated with angular cheilitis, glossitis, and burning sensation, although a great number of patients do not refer any symptom^{1,17}.

The aetiology of DS is not yet well understood, being associated with local factors such as poor oral hygiene and mechanical trauma mainly caused by inadequate conditions of the prosthesis, as well as the presence of microbial agents. The commonly related microorganisms are yeasts of the *Candida* genus^{4,5,6,14,16,20}. Bacteria are also

reported as casual agents of these lesions, identified in general as Gram-positive cocci^{7,9}.

Differences between the oral flora from patients with and without prosthetic stomatitis have not been observed, but the plaque index and the frequency of yeasts are higher in the first group, although their specific role is not yet well established⁷.

Virulence factors, like proteinase and phospholipase produced by *C. albicans*, have been also evaluated^{12,15}, since they are prone to facilitate adhesion, conditioning a more pathogenic activity^{3,13}. These enzymes are capable of invading tissues, leading to dysfunction or even rupture of cellular membranes, and to a higher grade of adherence and colonization^{2,8,12,15}.

BUDTZ-JORGENSEN³, comparing *C. albicans* samples from patients with and without prosthetic stomatitis, suggested that the pathogenicity of the yeasts could be related to the enzymatic activity,

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even when an association between the proteolytic activity *in vitro* and the severity of the lesions has not been observed³. Proteinase production can also interfere in the pathogenic potentiality of bacterial components of plaque through cleavage of salivary antibacterial immunoglobulins¹⁸. Having all these aspects in mind, the aim of this study was to assess edentulous patients, with and without DS, evaluating the frequency and species of yeasts related to both groups, as well as the proteinase and phospholipase levels.

MATERIAL AND METHODS

From 120 patients from the School of Dentistry, University São Paulo, 69 were selected for this study, being the main inclusion factor, the edentulous maxilla. Patients of both genders varying from the 3rd to the 8th decade of age (mean age: 59.23 years; standard deviation: + 13.91) were selected. From all patients, 49 were denture wearers presenting DS on the palate, while 20 were totally edentulous patients without DS. A specific protocol was utilized considering age, previous and present medical history, as well as other information like time of denture usage, frequency of oral and prosthesis hygiene and reported symptoms. Extra- and intra-oral examinations were performed.

Using sterile swabs, samples for the mycological test were collected from the hard palate, and immediately inoculated on plates of Sabouraud dextrose agar* with 100 µg/ml of chloramphenicol**. Incubation was made at 25°C, up to 15 days. Colonies suspect of being *Candida spp* were subcultured on the slant and maintained at 25°C, with successive replications until identifica-

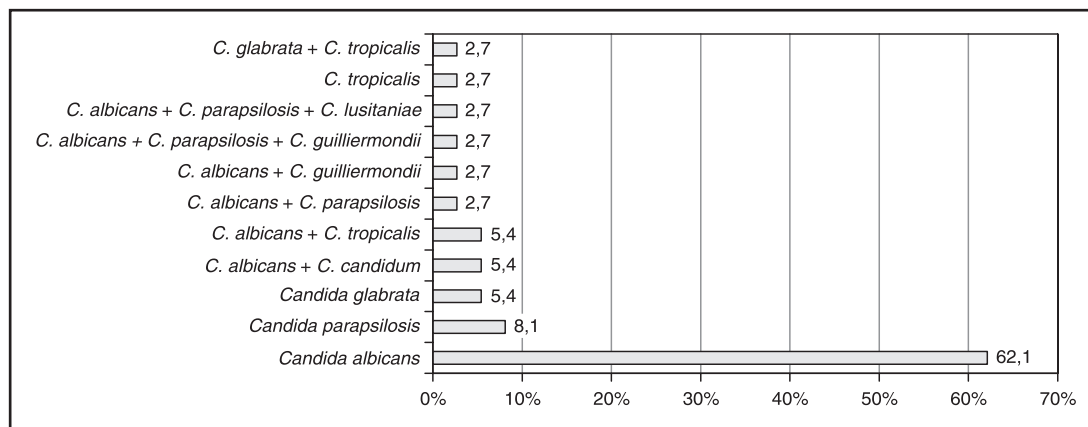
tion. The colonies were identified according to the methodology described by KREGGER-VAN RIJ¹⁰ and submitted to enzyme production assays.

The study of proteinase was carried out based on RUCHEL *et al.*¹⁷, and the study of phospholipase, on PRICE *et al.*^{15,16} and WILLIAMSON *et al.*²⁰, being the lecture performed after 72 hours. The samples producing enzymes presented an opaque zone of precipitation around the yeast inoculation point, being the enzymatic activity obtained by measuring the colony diameter and dividing it by the diameter plus the precipitation zone (P_z). In both cases, when P_z was 1.00, the enzymatic activity was zero. Between 0.64 and 0.99, this activity was considered positive; when lower than 0.64, it confirmed a strong positivity.

RESULTS

From sixty-nine patients, 62.3% (43) presented positivity for yeasts. In the group with DS, 75.5% (37/49) of the patients had yeasts isolated, being 81,1% of the samples (30/37) identified as *Candida albicans*. In seven patients, it was possible to identify *C. parapsilosis*, *Geotrichum candidum*, *C. tropicalis*, *C. guilliermondii*, and *C. lusitaniae* associated with *C. albicans*, besides other isolated species such as *C. parapsilosis*, *C. tropicalis* and *C. glabrata* (Graphic 1).

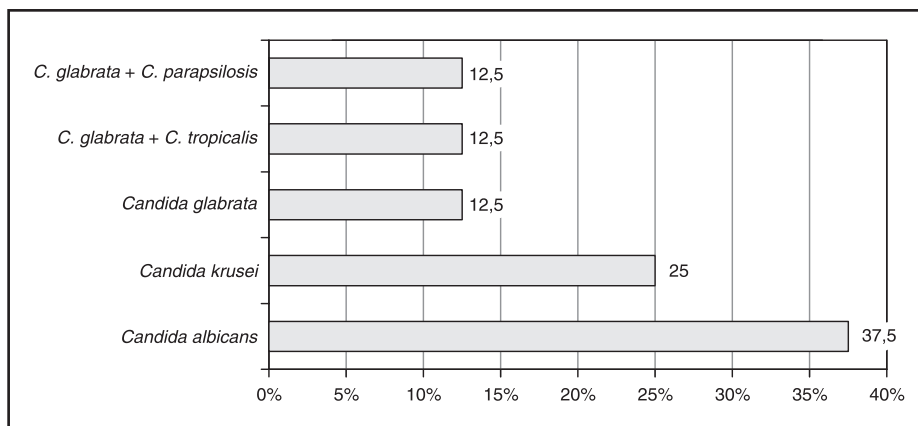
The collected material from the group without lesions demonstrated that eight samples were positive for yeasts, corresponding to 40% (8/20). *C. albicans* was identified in 37.5% (3/8); *C. krusei*, in 25% (2/8); *C. glabrata*, in 12.5% (1/8), and also associations such as *C. tropicalis* and *C. glabrata*; *C. parapsilosis* and *C. glabrata*, each one in different samples (Graphic 2).



GRAPHIC 1 - Frequency of yeasts in patients with denture stomatitis.

* Difco.

** Chloromicetin – Parke Davis.



GRAPHIC 2 - Frequency of yeasts in patients without denture stomatitis.

The enzymatic evaluation demonstrated that 100% (30/30) of *C. albicans* colonies produced proteinase, presenting a strong positivity. The enzymatic production of phospholipase was present in 83.3% (25/30) of the samples, with a strong positivity in 36.6% of them.

DISCUSSION AND CONCLUSION

The aetiology of DS is dependent on varied factors, including the usage of dentures and the association with yeasts as well as with bacteria, besides the presence of microtraumas and poor oral hygiene. The establishment of this condition is mainly related to the involvement of yeasts. An adequate diagnosis is important to prevent more aggressive or diffuse candidosis, when other predisposing systemic factors are present^{6,12,18}.

A higher frequency of isolation of yeasts in patients presenting DS has already been observed^{6,12} and it was confirmed in this study, which revealed positivity for yeasts in 75.5% of the sample.

Various species of yeasts were identified. The *C. albicans* species corresponded to 62.1% of the yeasts isolated from the patients with DS. Identification of more than one species per clinical sample was observed in relation to *C. albicans*, *C. glabrata* and *C. tropicalis*, as quoted by VANDENBUSSHE; SWINNE¹⁹.

In our results, eight different associated species were identified, similarly to the results of KREHER *et al.*¹¹, that reported 27% of associations in their sample.

Isolation of yeasts was also positive in edentulous patients without DS. *C. albicans*, *C. krusei*, *C. glabrata* and *C. tropicalis* were identified

in association with *C. glabrata*, which are results similar to those of BUDTZ-JORGENSEN⁶, although *C. tropicalis* and *C. guilliermondii* were not observed, suggesting that a variability of species can occur also in totally edentulous patients without DS.

In relation to the enzymatic activity of *C. albicans*, we have to consider the high level of proteinase production in 100% of them. BUDTZ-JORGENSEN³ did not find a positive correlation between the *in vitro* proteolytic activity and the severity of the lesion. Since they used different techniques from the ones employed in this study, it is difficult to establish comparisons. The high level of proteinase production suggests that this enzyme can play a role in the establishment and maintenance of DS. More investigations are necessary to help to understand the relationship between its action in the mucosae and its possible influence on the clinical aspects of DS.

Differently from proteinase, phospholipase presented a low or intermediate level of production, not suggesting a defined activity related to the clinical aspects observed.

So, *C. albicans* was the most frequent yeast in complete denture wearers presenting DS, compared with patients without DS. Associations of yeasts could be observed, including different species. The proteinase from *C. albicans* presented a high level of positive production, differently from phospholipase.

Candidosis is still undefined in some aspects, although it is frequently observed in denture patients. The presence of yeasts and their enzymatic activity need to be further investigated.

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A Estomatite Protética (EP) é freqüentemente observada em pacientes portadores de prótese total, sendo a presença de fungos considerada um importante fator para o seu aparecimento. Baseado neste fato, avaliamos pacientes edêntulos com e sem estomatite protética, identificando os fungos presentes, e os níveis de proteinase e fosfolipase produzidos por *Candida albicans*. Os resultados mostraram que *C. albicans* foi a espécie mais freqüente, prevalecendo em pacientes com EP. Todas as cepas de *C. albicans* isoladas foram fortemente positivas para proteinase, diferentemente da atividade de fosfolipase.

UNITERMOS: *Candida albicans*; Estomatite sob prótese; Proteinase; Fosfolipase.

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