Candida albicans: Frequency and characterization in oral cancer (Stage I) from smokers and drinkers

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Summary

The frequency and the biotype of *Candida albicans*, from patients with epidermoid carcinoma of the oral mucosa (stage I) were evaluated. The patients chosen were habitual drinkers and smokers, aged 34 to 81 years who had not submitted previously to any treatment. They exhibited ulcero-vegetative lesions, mainly on the floor of the mouth, palate and tongue and were classified as stage TNM 100 - TNM 200. Samples from the buccal mucosa were collected for mycological study including: identification of yeasts, serotyping, determination of exo-enzymes as proteinase and phospholipase as well as "killer" assay for biotype characterization. Positive cultives for yeasts were observed in 51.5% of the patients(17/33), being 21.2% represented by *C. albicans*, all serotype A. The "killer" test demonstrated two different biotypes of *C. albicans*, namely 211(71.4%) and 611(28.6%), with high levels of proteinase (Prz < 0.30), while phospholipase presented intermediary levels (Pz > 0.29 and < = 0.69). These data suggested a potentiality to virulence of *C. albicans*, although did not show an association of a particular biotype with the carcinogenic factors present or with the development of oral epidermoid carcinoma in this initial stage.

Key words Oral cancer, Proteinase, Phospholipase, Killer factor, Biotypes, *Candida albicans*

Candida albicans: frecuencia y caracterización en cancer bucal (Estadio I) de tabaquistas y bebedores habituales

Resumen

Fueron evaluados, la frequencia y los biotipos de *Candida albicans* aislados de pacientes portadores de carcinoma epidermoide en la mucosa bucal (Estadio I). Los pacientes eran tabaquistas y bebedores habituales, con edades entre los 34 y los 81 años. Ninguno de ellos habia sido tratado previamente. Todos exhibían lesiones ulcero-vegetantes clasificadas como TNM100 - TNM200. Fueran recogidas muestras de la mucosa bucal para su estudio micologico, siendo realizados: identificacción de *C. albicans*, serotipificación, evaluacion de exoenzimas (proteinasas y fosfolipasas), asi como el factor *killer*, con vistas a la obtención de biotipos.

Los resultados demostraron que el 51,5% de los cultivos presentaban colonias de hongos, siendo en el 21,2% para *C. albicans*, serotipo A. El factor *killer* demostró la presencia de dos diferentes biotipos de *C. albicans*, respectivamiente el 211 (71,4%) y el 611 (28,6%), con alta producción de proteinasas y producción intermediaria de fosfolipasas. Los datos obtenidos sugieren una potencialidad de virulencia de *C. albicans* aunque no permitieron una asociación de un biotipo específico con los factores carcinogénicos presentes, para el desarollo del carcinoma epidermoide en el estadio considerado.

Palabras clave Cáncer oral, Proteinasa, Fosfolipasa, Factor *killer*, Biotipos, *Candida albicans*

The scarcity of researches reported on the literature related to patients with oral cancer at initial phase and without any therapeutical procedure going on has called our attention. Some evidences were presented in relation to species of the genus *Candida*, which could play a role on the development of oral cancer [1], since filamentous forms mainly of *C. albicans* were associated to epithelial maturation disorders [2], leukoplakia or precancerous lesions [3]. Therefore an evaluation of the frequence and some characteristics of *C. albicans* of patients with oral cancer in early stage was performed, being those represented by T_1NoMo to T_2NoMo, not submitted to any treatment. The patients should be drinkers and smokers since these agents as well as yeasts like *C. albicans*, are prone to favour the production of nitrosamines, which could be an adjuvant of neoplasia development.

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CASUISTIC

Forty-five patients with Epidermoid carcinoma of the mouth (EC), classified clinically as T_1NoMo or T_2NoMo, confirmed by histopathology were examined according to an established protocol, in which patients who have received any previous treatment for the disease, or even under antibiotic and / or corticosteroid therapy were not included.

MATERIALS AND METHODS

Material was collected from the buccal mucosa of 33 patients and cultured outside the neoplastic area, being collected by only one examinator through a sterile swab. The material was cultivated in Petri dishes with agar Sabouraud dextrose, plus chloramphenicol, for at least 15 days at 25°C.

The different strains of *C. albicans* were selected and isolated according to colour, size, consistence, surface, borders, brightness and colony topography. The representative morphological types were sampled in the same medium. The yeasts colonies were identified according to Lodder [4] and Kreger van Rij [5], utilizing the protocol from the Yeasts Section of the Department of Microbiology (ICB - USP São Paulo, Brazil).

Serotyping. Specific antiserum: total antiserum was obtained by innoculation of rabitts with serotype A *C. albicans*, ICB-USP 12. Specific anti-A antiserum was prepared adsorbing the total antiserum with *C. albicans* serotype B ICB-USP 156 cells, according to Hasenclever and Mitchell [6].

Slide agglutination technique. The pattern of serological reaction was determined using the slide agglutination test. A drop of the specific antiserum was placed on a microscope slide, mixed to an equal amount of a suspension of the studied strain (10^6 cells/ml) in formalized saline solution and turbidimetrically adjusted with Mac Farland scale, to be similar to 10 tube. The positive agglutination reaction classified the strain as serotype A. Otherwise the strain was classified as serotype B.

Phospholipase. Phospholipase activity was performed through a plate assay, according to Price *et al.* [6]. After inoculation at 37°C for 24 h, the plates were analyzed and the phospholipase activity (Pz) was determined by the ratio between the diameter of the colony and the total diameter of the colony plus the precipitation zone. Thus, Pz = 1.00 means that the isolate was phospholipase negative. Pz < 1.00 and > 0.69 means that the tested strain was low positive. Where Pz < 0.69 > 0.30 means that the tested strain was positive (intermediate) and Pz < 0.30 means that the strains was strongly positive.

Proteinase. Proteinase activity production was determined as described by Rüchel *et al.* [8]. The plates were incubated at 37°C for 2 to 3 days; the production of a clear ring around the colony was taken as positive proof of proteinase production. Total proteolysis was visualized through staining with amido black (1 gL^{-1} in 3.5M acetic acid and subsequent differentiation in 1.2 M acetic acid). The precipitation zone (Prz) indexes were determined the same manner as described for phospholipase by Price *et al.* [7].

Killer typing. The killer typing was done according to Polonelli *et al.* [9]. *C. albicans* strains were cultivated at 25oC for 24 h. The cultures were diluted in 10 mL of pH 4.00 YEPD medium and after, 1mL suspension was mixed to 20 mL of pH 4.5 YEPD agar, preserved at 46oC. The medium was placed into Petri dishes and left to solidify. Standard killer producer strains, respectivelly Stum 1034-k1 - *Pichia* sp.; Stum 1035-k2 - *Pichia* sp; UM - Milan University, Milan, Italy - k3 - *Pichia anomala*; CBS - Centralbureau Voor Schimmelcultures, Bearn, The Netherlands; 5759-k4 - *Pichia anomala*; Ahearn UN 866-k5 - *Pichia anomala*; Ahearn WC 40-k6 - *Pichia californica*; Ahearn WC 41-k7 - *Pichia californica*; Ahearn W44-k8 - *Pichia dimennae*; Ahearn 51-k9 - *Pichia mrachii*, were radially applied into nine equidistant points. The plates were incubated at 25°C for 72 h. Cells could die by the toxin effect stained in blue, or produced an inhibition halo around the killer strains. The numerical code proposed by Polonelli *et al.* [9] was utilized for typing.

RESULTS

Seven of the 33 patients presented positive cultures for *Candida albicans*, being serotype A obtained in all these samples. Other species and genera were also found isolated or in association and were represented by the following species in nine patients: *C. tropicalis, C. glabrata, C. parapsilosis, Trichosporum beigellii, Cryptococcus laurentii*, and *Rhodotorula rubra*, as predominant (Table 1).

Table 1. Main genera and species of fungi isolated from the oral cancer patients.

Genera and specie	No. of isolates
Candida albicans	7
Candida tropicalis	2
Candida glabrata	1
Candida parapsilosis	1
Trichosporum cutaneum	1
Cryptococcus laurentii	1
Rhodotorula rubra	4

Proteinase and phospholipase activity by *C. albicans* were represented by high levels of production for the first and an intermediate for the second (Table 2).

Table 2. Distribution of *Candida albicans* biotypes from oral cancer patients after the "Killer" test.

Biotype	No. of isolates
211	5
611	2
Total	7

The sensitivity for Killer toxins, identified Strain 211 in 71.4% and 28.6% of strain 611 (Table 3).

Table 3. Proteinase (Prz) and phospholipase (Pz) indexes of the *Candida albicans* isolates.

Patient	Proteinase (Prz)	Phospholipase (Pz)
01	0,16	0,72
02	0,17	0,58
06	0,21	0,70
08	0,28	0,66
10	0,20	0,69
11	0,26	0,81
28	0,26	0,75
Average	0,21	0,69

DISCUSSION

The importance of the protocol established for this study was based on the idea that the immunosupression stage of the studied patients was different from those in advanced stages of neoplasia or under radiotherapy treatment. These variables can modify the host-parasite relationship, leading to different clinical and microbiological features allowing an increase of the pathogenic load and virulence of the yeasts present in the mouth of these patients.

Our results in relation to *C. albicans* isolation, were different from those of Krogh [10] in leukoplakia or even from oral cancer patients in more advanced stages or during therapy [11,12], and similar to other studies of head and neck cancer patients [13].

None of our patients had clinical signs of candidosis, even those utilizing full dentures which can induce a higher susceptibility of erythematous candidosis. Serotype A was predominant and can be found in other reports of cancer as well in AIDS patients [12,14].

The killer biotype identified in the great majority as strain 211, was also observed in samples of the oral mucosa of HIV+ patients and in healthy carriers, not indicating any special killer biotype of *C. albicans*, which suggests that it can coexists harmoniously on the oral cavity of healthy carriers [16].

Phospholipase and proteinase indexes were positive presenting the first intermediate levels comparable to healthy carriers, while high levels of proteinase indicated some modifications possibly related to virulence, since this can be considered an important factor of pathogenicity. Healthy or diabetic patients without clinical signs of candidosis did not present such levels although similar high indexes were observed in healthy patients with erythematous candidosis of the palate, associated to the use of full dentures [15-17].

We could conclude that the establishment of a particular biotype of *C. albicans* could not be determined in this stage of oral cancer development in face of the present carcinogenic factors.

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