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Oral *Candida* species in head and neck cancer patients treated by radiotherapy

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ABSTRACT

Objective: This paper aimed to identify and quantify *Candida* on head and neck irradiated patients with two comparative elderly populations.
Materials and methods: Saliva was sampled from 29 head and neck irradiated patients (group 1) 34 non-institutionalized elderly patients (group 2) and 29 institutionalized elders (group 3) and matched by age. For quantification, the obtained saliva was sewed on CHROMagar *Candida*®, which was used also for presumptive identification of *Candida*, API 20C AUX®, microculture and RAPD (OPE-18) were used for the final identification.
Results: Among the 92 patients surveyed, 51 (55.4%) had scores classified as positive for *Candida*, and among individuals of groups 1 and 3, 58.6% had *Candida*. There was higher positive/carrier conditions (CFU/sample) in groups 1 and 3, comparing with group 2 ($P = 0.011$). Gender was not associated with *Candida* portability ($P = 0.334$). *Candida* portability was significantly associated with the presence of candidosis ($P = 0.031$) and xerostomic state ($P = 0.007$). The isolates and definitive confirmed colonies were *Candida albicans*, *Candida dubliniensis*, *Candida tropicalis*, *Candida krusei*, *Candida glabrata*, *Candida parapsilosis*, *Candida guilliermondii*, *Candida lusitaniae*, and *Candida kefyr*. Among the species identified, *C. albicans* was the most frequent, followed by *C. tropicalis*, *C. parapsilosis* and *C. glabrata*. *C. albicans* was more prevalent in group 2 and 3, 45% and 41.2%, respectively, whereas, the most prevalent species in group 1 were *C. tropicalis* (27%), *C. albicans* and *C. parapsilosis* (16.2% for both). On multiple statistical models, only radiotherapic treatment was associated with positiveness to *Candida*.
Conclusions: Diversification and major prevalence of non-*albicans* species were observed in irradiated patients on head and neck and this treatment is straightly associated with fungus positiveness.

1. Introduction

Candidosis is an important oral condition associated with immunossupressive status, oncologic treatment inducing xerostomic state, mucositis and neutropenia, microbial imbalance and prosthesis use [1–3]. It is frequently found in elderly populations being associated with chronic conditions, pharmacological controls and even with physiological effects of aging [4,5]. Elderly patients with head and neck malignancies, submitted to conventional radiotherapy are the one of the preferential targets to *Candida* overcolonization due to induced xerostomic state, concomitant mucositis and chemotherapical care, and difficulties to establish an adequate oral care [6–9].

Several papers previously focused on *Candida* colonization on head and neck irradiated patients [1,3,6–8,10–13]. Some of them showed *Candida* quantification and identification, but sometimes presumptively [12,13]. Rare studies focused on irradiated patients on head and neck had epidemiological approach using RAPD methodology [14,15]. That is, an analysis with comparative groups matched by age, including differential oral self-care and socioeconomic status lacks in current literature [16]. With this proposal, this study aimed to quantify and identify *Candida* using RAPD methodology on head and neck irradiated patients with two comparative elderly populations.

2. Materials and methods

The isolates of *Candida* used in this study were collected from salivary collections of head and neck irradiated patients' oral cavity due to malignant neoplasm ($n = 29$) (group 1) and elderly voluntaries, being 34 non-institutionalized (group 2) and 29

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institutionalized (group 3), matched by age. The collection comprised 91 isolates of *Candida* species.

2.1. Data collection

Patients belonging to group 1 were submitted to conventional radiotherapy with tumoricidal doses on cervicofacial fields with daily doses from 180 to 200 cGy, due to malignant neoplasm on head and neck. As selection criteria, patients who were antifungal or broad-spectrum antimicrobial users, those who were under concomitant chemotherapy treatment or those who did not agree to take part in this study did not participate. General clinical data obtained from the analysis of medical records and oroscopical examinations were registered in clinical records. Data collection about personal information, about cancer, radiotherapic features, drug uses, total radiated fields, and side effects of radiotherapy were done, including xerostomic state, which was assessed by asking the individuals if they had dry mouth or not. Oral candidiasis was evaluated by a professional calibrated and the mucositis using a scale of oral of World Health Organization that were verified by oroscopy. Personal and clinical characteristics of the group were shown in Table 1. Thirty-four elderly subjects without malignant conditions and predictors of xerostomic state, as chronic predisposal illness and hypossalivation induced by drugs, were included in this study (group 2). The same characteristics were observed on a third group of 29 elderly residents of a nursing home (group 3). Salivary collections with a sterile swab was made on group 1 during 10th to 23rd sections of radiotherapy

2.2. Isolation and presumptive identification of Candida species

The isolation of yeasts was made from salivary samples collected on the buccal mucosa and tongue with a swab and sterile saline solution (NaCl, 0.85%), as diluent. The isolation and presumptive identification was made by withdrawing aliquots (100 μL) of each sample and placing on plates containing CHROMagar *Candida*® and incubated at 37 °C for 24–48 h, on duplicates. Yeast identification was made by considering the morphology and color of the colonies [13]. Each colony of *Candida* was cataloged and then stored at −20 °C in Sabouraud Dextrose Broth (DSB, Oxoid, England) amended with glycerol (40%, v/v). As quality control (QC), ATCC 10231 of *Candida albicans* was used.

2.3. Microculture characterization of Candida

Microcultures with Cornmeal Agar-Tween 80 (Rheum, Lenexa, KS-CTA, USA) were made to highlight blastospores, chlamydospores, pseudohyphae and true hyphae of the isolates. To differentiate *C. albicans* and *Candida dubliniensis* from other *Candida* species, germ tube production was viewed on bovine serum [17,18]. To distinguish *C. albicans* from *C. dubliniensis*, cultivation on Sabouraud Dextrose Agar (Oxoid, England) for 48 h was made in 42 °C, using ATCC 10231 as QC.

2.4. Identification by the API 20C AUX®

The inoculum used for this procedure was obtained from cultured yeast on Sabouraud Agar. The procedures for inoculation and interpretation were performed according to the panel provided from manufacturer (BioMerieux, France). Identification list on this index was considered as excellent (% ID $\geq$ 99.9, $T \geq 0.75$), very good (% ID $\geq$ 99.0 and $T \geq 0.5$) or acceptable (% ID $\geq$ 90.0 and $T \geq 0.5$) [19].

2.5. Identification of isolates by RAPD (random amplification of polymorphic DNA)

The extraction and purification of DNA from isolates of *Candida* spp. was made with the kit Purelink Genomic DNA® (Invitrogen K1820-02, Brazil). The DNA used was obtained from cells grown in YPD broth (1% Malt Extract Powder, 2% bacteriological peptone and 2% dextrose-D-glucose) at 37 °C shaking (150 rpm, 24 h) [14]. A total of 50 μL of concentrated suspension of each isolate, obtained by centrifugation (3500 rpm, 30 min). The purification of DNA was made by adding 200 μL of Digestion Buffer, 20 μL Proteinase K and 20 μL RNase. We added it to 200 μL of Binding Buffer and then the tubes were heated for 10 min at 80 °C on water bath. To neutralize the detergent and to allow the connection with the silica column, 200 μL of absolute ethanol was added (Merck,

Table 1
Distribution of gender, age, xerostomic state and candidosis on all groups and location of the tumor and mucositis on individuals by group 1.

Variable	Contrast	Group		
		Group 1 $n = 29$	Group 2 $n = 34$	Group 3 $n = 29$
Gender	Male	23 (79.3%)	21 (61.8%)	7 (24.1%)
	Female	6 (20.7%)	13 (38.3%)	22 (75.9%)
Age	Mean (median)	61.8 (61)	66.4 (64.5)	73.7 (74)
Xerostomic state	Yes	18 (62.1%)	4 (11.8%)	7 (24.1%)
	No	11 (37.9%)	30 (88.2%)	22 (75.9%)
Clinical candidosis	Yes	12 (41.4%)	4 (11.8%)	2 (6.9%)
	No	17 (58.6%)	30 (88.2%)	27 (93.1%)
Site of tumoral lesions	Pharynx	7 (24.1%)	NA	NA
	Oral floor	3 (10.3%)	NA	NA
	Tongue	3 (10.3%)	NA	NA
	Gingival border	5 (17.2%)	NA	NA
	Larynx	2 (6.9%)	NA	NA
	Facial skin	4 (13.8%)	NA	NA
	Other sites	5 (17.2%)	NA	NA
Mucositis	0	11 (37.9%)	NA	NA
	I	1 (37.9%)	NA	NA
	II	14 (48.3%)	NA	NA
	III	3 (10.3%)	NA	NA

NA, not evaluated variable to the group.