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Radiotherapy effect on frequency of *Candida spp.* and on virulence of *C. albicans* isolated from the oral cavity of head and neck cancer patients

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ABSTRACT

The aim of this study was to investigate the diversity and prevalence of yeasts, and the virulence of *C. albicans* found in the oral cavity during the course of ionizing radiation treatment of patients with head and neck tumor (HNTP). Samples from 21 HNTP and 24 healthy controls were isolated and identified. *C. albicans* isolated from two patients during radiotherapy were analyzed for virulence factors. Radiotherapy induced a higher level of both yeast colonization (81% vs 33%) and non-albicans *Candida* (NAC) colonization (52.4% vs 4.0%) in HNTP than the control group. Patients were colonized by 5 different NAC species: C. glabrata, C. tropicalis, C. parapsilosis, C. krusei and C. kefir. On the other hand, *C. albicans* colonization was similar in patients and controls (6/21, 28.6% vs 7/24, 29.2%, respectively). Also, of the 11 patients assessed before and during radiotherapy, 5 (45.5%) were colonized before the start of treatment and another 5 (45.5%) during treatment. All of the latter were colonized by NAC species alone. Moreover, we observed a significant and continuous enhancement of *C. albicans* virulence as the radiotherapy progressed, in the two patients involved in this test. Thus, it is concluded that radiotherapy is an important predisposing factor for the oral candidiasis, including NAC species. Also, it may facilitate the development of more virulent *C. albicans* strains.

Keywords: *Candida*. Radiotherapy. Virulence factor. Oral candidiasis.

INTRODUCTION

Candida albicans and related species are opportunistic pathogens, which normally live as commensal organisms in various parts of the human body (Soll, 2002), but, when the immune system is down-regulated, can infect cavities and tissues of the host (Umazume et al., 1995). Several studies in cancer and AIDS patients have shown a high prevalence of colonization of the oral mucosa by *Candida* spp., especially *C. albicans*, but also by various non-albicans *Candida* (NAC) species (Vazquez et al., 1998; Redding et al., 2000).

The goal of radiotherapy is to destroy malignant cells, but it may also cause injury to normal cells, which can persist after treatment (Garfunkel, 2004; Wright et al., 2005). Besides mucositis, exposure of the head and neck to ionizing radiation affects the natural immunological barriers of the oral cavity, leading to enhancement of morbidity and, in some cases, to a higher risk of systemic infection (Bufarah, 2002; Sonis, 2004). Patients with head and neck cancer subjected to radiation treatment also have a higher risk of oral colonization by *C. albicans* and NAC species (Ozcelik et al., 2004; Thaweboon et al., 2008). *C. albicans* is still the most virulent species found in immunocompromised patients (Paula et al., 1990; Marodi et al., 1991). Nevertheless, longitudinal studies on the effects of radiotherapy on the diversity, prevalence and virulence of *Candida* spp. are scarce in the literature (Jham et al., 2007). Therefore, this study was designed to investigate the diversity and frequency of *Candida* species in the mouth before and during ionizing radiation treatment of patients with head and neck tumor (HNTP), as well as to determine whether this treatment may induce an enhancement of *C. albicans* virulence.

MATERIAL AND METHODS

Samples

Yeasts were isolated from 21 HNTP, diagnosed histopathologically and treated at Santana Radiotherapy Clinic in Maringá, PR, Brazil. Samples were taken, with informed consent, by mouth washing with 10 mL sterile water, as described by Bergbrant & Faergemann (1997). Eleven of these patients had samples collected before and weekly throughout the radiation therapy, as presented in Table 1. A control group comprised 24 healthy Pharmacy academics not involved with the hospital environment or clinical laboratories. For each patient and control subject a file was opened, recording gender, age and the number,

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level and localization of radiation treatments. This study had prior approval of Maringá State University's Human Research Ethics Committee.

Table 1. *Candida* species isolated from the oral cavity of 11 head or neck cancer patients before and during radiation therapy.

	Time Of Treatment In Weeks								
Patient	0	1	2	3	4	5	6	7	8
5	C.g	C.g	C.g*						
12	C.a		C.a	C.a	C.a	C.a[f]			
13					C.t		C.t		[f]
14			C.p						[f]
15		C.g	C.g	C.g	C.t	C.t/C.g[f]			
16	C.p	C.p	C.p			[f]			
17	C.t		C.t	C.t		[f]			
18	C.g	C.g	C.g					[f]	
19						[f]			
20	C.a	C.a	C.a	C.a	C.a	C.a	C.a	C.a	C.a[f]
21		C.p				[f]			

0 = before start of radiotherapy treatment; *= patient died during treatment; [f]= end of treatment. C.g.= *Candida glabrata*, C.a.= *Candida albicans*, C.t.= *Candida tropicalis*, C.p.= *Candida parapsilosis*

Culture and identification of the yeasts

Oral washes from both patients and controls were centrifuged at 1170 xg for 10 min, and 30µL of the sediment was plated on Sabouraud Dextrose Agar (SDA) (Difco, Becton Dickinson, Sparks, MD, USA), supplemented with 100µg/mL chloramphenicol, and incubated at 25ºC for 7 days. The positive samples were plated on chromogenic medium (CHROMagar™ *Candida*) (CHROMagar Microbiology, Paris, France). Yeasts were identified by classical methods: germ tube production, micromorphology and chlamydospore production on Tween 80-cornmeal agar, assimilation and fermentation tests, as described by Larone (1995), complemented by Kurtzman and Fell (1998). All isolates identified as *C. albicans* were screened for their ability to grow on SDA at 45ºC for 48h, to exclude *C. dubliniensis*.

Determination of antifungal drug Minimal Inhibitory Concentration

Minimal inhibitory concentration (MIC) of fluconazole (Galena Química Farmacêutica, Brazil), nystatin (Sigma), amphotericin B (Fungizon®, Bristol-Myers Squibb Brazil), itraconazole and ketoconazole (Janssen Pharmaceutical, Titusville, NJ, USA) were determined by the broth microdilution method specified by the Clinical and Laboratory Standards Institute (CLSI, document M27-A2, 2002). Stock solutions were prepared at 10 times the final concentrations and diluted with RPMI 1640 (Sigma, Germany) containing L-glutamine but not bicarbonate, supplemented with 2% dextrose and buffered to pH 7.0 with 0.165M of N-morpholinopropanesulfonic acid (MOPS), to obtain twice the final concentration. Each column of wells on the microplate received a series of increasing concentrations of the drugs, ranging from 0.125 to 64 µg/mL (fluconazole and nystatin) and from 0.03 to 16 µg/mL (itraconazole, ketoconazole and amphotericin B). For each isolate, negative and positive controls were included. On each microplate, a strain of *Candida parapsilosis* (ATCC 22019) was included as the reference yeast. The plates thus mounted were incubated at 35°C for 48-72 h with daily monitoring. After 48 h, the plates were read in a microplate reader (Asys Hitech GmbH, Eugendorf, Austria). The minimal inhibitory concentrations (MIC) of the azoles and the polyenes were taken as the lowest concentration of the drug capable of inhibiting 50% and 90%, respectively, of the growth of each yeast, relative to its positive control.

Frequency and size of *C. albican*s germ tubes

A yeast suspension was prepared from a fresh yeast culture on SDA (18-24 h) in 1mL sterile water, at a density adjusted to the fourth tube of the McFarland scale. The suspension was centrifuged at 1170 xg for 10 min. and the sediment resuspended in 0.5 mL of RPMI 1640 medium (Gibco, Grand Island, NY, USA), supplemented with 50% heat-inactivated human serum, before incubation at 37ºC for 3 h. The culture was then resuspended vigorously for 30s and examined under an optical microscope with a reticule in the eyepiece. The frequency and the length of the germ tubes of the samples were determined in blind tests and the results were expressed as average ± S.D. of three independent experiments.

***C. albicans* phagocytic index and polymorphism in the presence of mouse peritoneal macrophages**

These experiments were performed by a modification of the method of Andrade & Felipe (1992), using peritoneal macrophages collected from male Swiss mice aged 6 to 8 weeks. Immediately after mice anesthesia induced with 50 mg/kg ketamine hydrochloride (Ketamin, Cristália Ltda, Brazil) + 50 mg/kg thiazine hydrochloride (Rompum, Bayer SA, Brazil), cells from the peritoneal exudates were collected in two washes with 3 mL cold phosphate-buffered saline (PBS). The cells were counted and made up to $2x10^6$ cells/mL in cold RPMI 1640 medium supplemented with 10% fetal calf serum (Gibco, Grand Island, NY, USA). Next, 100 µL of this suspension was plated into wells of a 96-well sterilized microplate, and incubated at 37ºC for 2 h in 5% CO_2. The non-adherent cells were washed out twice with supplemented RPMI 1640 and counted, to determine the number of adherent cells remaining in the wells. The adherent macrophages were then cultured at 37ºC for 48hs under 5% CO in supplemented RPMI 1640, for cell adaptation, the medium being replaced after 24hs. Cell viability was determined by Trypan blue staining and all the experiments were performed with cell viability greater than 95%. Next, 100 µL of *C. albicans* suspension was added to the wells at a density giving 2.5 yeasts / macrophage, and incubated for an additional 2hs at 37ºC in 5% CO_2. All *C. albicans* isolates from the same patient were tested with one pool of macrophages harvested from 3 to 4 mice. The culture of macrophage and *Candida* was then stained with 10 µL Neutral Red (10mg/mL in PBS; Sigma) for 5 min, before assessing the *C. albicans* polymorphism and *C. albicans* phagocytic index (PI) in 200 macrophages under an inverted optical microscope (PI = % of infected

macrophages x average number of *C. albicans* inside each macrophage). The results were expressed as average ± S.D. of three experiments.

C. albicans killing by mouse peritoneal macrophages

For these experiments, Swiss mouse peritoneal macrophages were cultured and incubated with *C. albicans* in 96-well microplates under the same conditions as in the preceding item. After incubation, the wells were washed vigorously 5 times with 200 µL of 0.05% Triton X in sterilized distilled water, and the washings collected in a tube. The complete removal of yeasts and macrophages from the wells was confirmed by inspection with an inverted optical microscope. Next, each sample was diluted 1:100 in PBS and 25 µL of this suspension was incubated on SDA for 48 h at 25ºC. After incubation, the colony-forming units (CFU) were counted and the proportion of *C. albicans* killed by the macrophages (1- (CFU with macrophages / CFU without macrophages)) was calculated. The results were expressed as average ± S.D. of three experiments.

Statistical analysis

Group means were compared by parametric (Student t, Tukey) and non-parametric (Student-Newman-Keuls) statistical tests, using the program SigmaStat 2.0 (Jandel Corporation, USA). Differences were considered significant when $P < 0.05$.

RESULTS

Overall data

All of the 21 cancer patients under study showed squamous cell carcinoma (SCC). The number, gender, age and localization of SCC and radiation dose of each patient are shown in Table 2. Radiotherapy induced a higher level of both overall yeast colonization (17/21, 81% vs 8/24, 33%) and non-*albicans Candida* (NAC) colonization (11/21, 52.4% vs 1/24, 4.2%) in HNTP than in the control group. On the other hand, *C. albicans* colonization of patients and controls was similar (6/21, 28.6% vs 7/24, 29.2%, respectively).

The distribution of the various *Candida* species among *Candida*-colonized patients and controls is presented in Fig. 1. Most *Candida*-colonized patients were colonized by at least one NAC species (11/17, 64.7%), against only 1 of the 8 colonized controls (12.5%). Five different NAC species colonized the patients: *C. glabrata, C. tropicalis, C. parapsilosis, C. krusei* and *C. kefir*. One patient was colonized by two NAC species (*C. glabrata* and *C. tropicalis*). On the other hand, most controls were colonized by *C. albicans* (7/8, 87.5%).

Table 1 presents data on *Candida* colonization throughout the radiation therapy. Five (45.5%) of the 11 patients that were monitored throughout the treatment were colonized by *Candida* spp. before the treatment started and another 5 (45.5%) were colonized at various times during treatment. Only one patient (9%) was free of oral *Candida* colonization throughout radiotherapy. Before radiation therapy, *C. albicans* and *C. glabrata* were the most frequent species (2 patients each), followed by *C. tropicalis* (1 patient). All strains isolated from patients colonized only after the start of treatment were NAC species: *C. parapsilosis* (n=3), *C. tropicalis* (n=1) and (from one patient) *C. glabrata* and *C. tropicalis*.

All *Candida* isolates obtained during the study were tested for antifungal drug susceptibility. MIC values ranged from 0.125 to 8µg/mL for nystatin and fluconazole; from 0.03 to 0.5 µg/mL for amphotericin B and itraconazole, and 0.03 µg/mL to 16µg/mL for ketoconazole. MIC values were similar for the strains isolated from patients and control subjects. Also, there were no differences in the susceptibility of the yeasts isolated before and during treatment.

Table 2. Patients' age, gender, diagnosis and radiation dose.

Patient n- age/gender	Diagnosis	Radiation (cGy)
1 - 44/M	SCC, vocal cord	7,200
2 - 38/F	SCC, hard palate	5,040
3 - 60/F	SCC, supraglottis	5,040
4 - 78/M	SCC, hypopharynx	7,200
5 - 58/M	SCC, retromolar	1,080[b]
6 - 54/M	SCC, larynx	5,040
7 - 52/F	SCC, parotid	6,300
8 - 42/M	SCC, supraglottis	5,040
9 - 53/M	SCC, larynx	7,200
10 - 70/M	SCC, larynx	7,200
11 - 60/M	SCC, floor of mouth	7,200
12 - 78/M	SCC, larynx	5,040
13 - 62/M	SCC, tongue	7,200
14 - 48/M	SCC, floor of mouth	7,200
15 - 58/M	SCC, inferior lip	5,040
16 - 69/M	SCC, larynx	5,040
17 - 33/M	SCC, floor of mouth	5,040
18 - 42/M	SCC, floor of mouth	6,300
19 - 50/M	SCC, larynx	5,040
20 - 52/M	SCC, larynx	7,200
21 - 48/F	SCC, mucosa jugal	5,040

SCC, squamous cell carcinoma
[b] patient died before treatment completed.
F= female
M= male

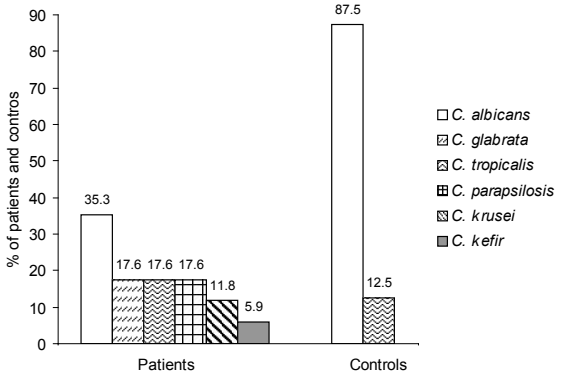

Figure 1: Distribution of patients and controls presenting different *Candida* species among *Candida* colonized patients (n=17) and controls (n=8). One patient presented two *Candida* species (*C. glabrata* and *C. tropicalis*) and is represented twice.

Effect of the radiation therapy on *Candida albicans* virulence

The two patients who presented *C. albicans* before and during treatment (patients 12 and 20) were followed

up, to check for alterations in *C. albicans* virulence factors induced by the radiation therapy.

A significant increase in the length of the yeast germ tubes was seen as treatment progressed (Fig. 2). Yeasts isolated from patient 12 showed a statistically significant increase from the second week of treatment, compared to yeasts isolated before treatment, while in patient 20 this increase was already seen in the first week. No difference was observed between sample 12, collected before treatment, and the average of the control group (11.2µm ± 2.50 versus 12.7µm ± 0.51, P=0.549). On the other hand, the average length of the germ tubes of yeasts from patient 20 was greater than those of the control group, even before the start of radiation therapy (15.2µm ± 0.79 versus 12.7µm ± 0.51, P=0.006). Regarding the frequency of germ tubes, only patient 12 presented a statistically significant increase during treatment (45.2% ± 6.25 before vs 56.5% ± 0.5 after treatment; P =0.003) (Fig.3). Although it was not statistically significant, there was also an increase in the frequency of germ tubes of yeasts from patient 20 (37.0% ± 6.06 vs 48.3% ± 7.37; P=0.182).

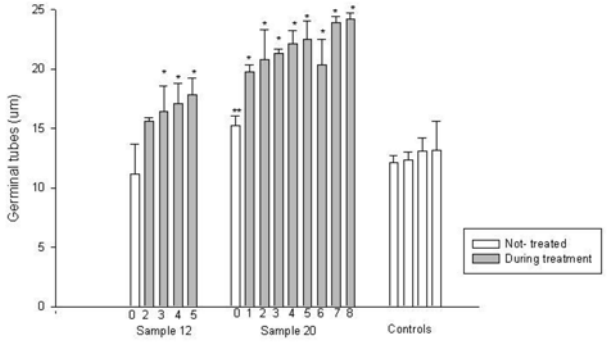

Figure 2: Average lenght of *C. albicans* germ tubes (micrometers) isolated from the oral mucosa of head or neck cancer patients 12 and 20 submitted to radiation therapy, and from unirradiated healthy controls. Number zero represents samples collected before treatment. The other numbers represent the time of treatment in weeks. The results are expressed as average ± standard deviation of 3 experiments. **P*<0.05 (when compared with samples before treatment); ***P*<0.05 (when compared with control samples).

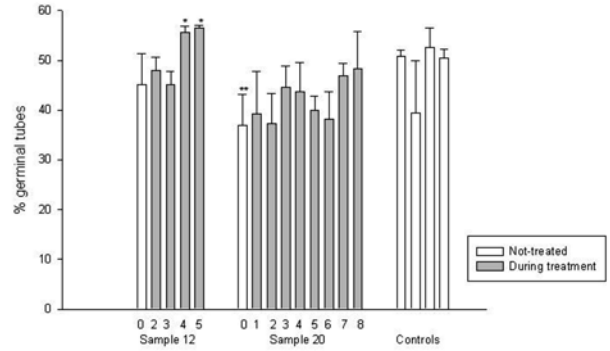

Figure 3: Frequency of *C. albicans* that developed germ tubes isolated from the oral mucosa of head or neck cancer patients 12 and 20 subjected to radiation therapy, and from unirradiated healthy controls. Number zero represents samples collected before treatment. The other numbers represent the time of treatment in weeks. The results are expressed as average ± standard deviation of 3 experiments. **P*<0.05 (when compared with samples before treatment); ***P*<0.05 (when compared with control samples).

C. albicans from patients 12 and 20 also exhibited a continuous and important enhancement of resistance to killing by mouse peritoneal macrophages, during treatment (Fig. 4). By the end of treatment, the yeasts from these patients roughly doubled their macrophage killing resistance (1.7 and 2.5 times, respectively). Again, no difference was observed between the killing resistance of yeasts collected from patient 12 before treatment and the control group, in contrast to yeasts from patient 20.

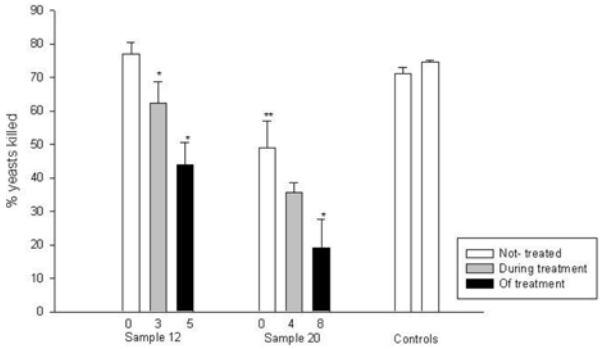

Figure 4: Percentage of yeasts killed by mouse peritoneal macrophages (1- (CFU with macrophages/ CFU without macrophages)), (2.5 yeasts/ macrophage), isolated alongside radiation therapy of head or neck cancer patients 12 and 20, and from unirradiated healthy controls. Number zero represents samples collected before treatment. The other numbers represent the time of treatment in weeks. The results are expressed as average ± S.D. of 3 experiments. **P*<0.05 (when compared with samples before treatment); ***P*<0.05 (when compared with control samples). CFU = colony-forming units.

We also tested the effect of radiation therapy on both the phagocytic index (PI) and the dimorphism of the yeasts inside the phagocytes. All *C. albicans* isolates were highly phagocytosed by mouse peritoneal macrophages (% phagocytosis before and at the end of treatment = 84.0 ± 8.48 and 81.5 ± 0.70 for patient 12 yeasts and 68.5 ± 2.12 and 78.5 ± 0.70 for patient 20 yeasts), but no change in their morphology or any tendency for PI to increase or decrease was observed during treatment.

DISCUSSION

Ionizing radiation applied to head and neck tumors during radiotherapy can affect oral mucosal cells, causing several problems for the patient, such as xerostomia and radiomucositis. These conditions may allow microbial infections, especially candidiasis, to become established (Sampaio et al., 1990).

Head and neck radiation treated patients in the present study show a higher level of yeast colonization than the control group. These data corroborate other papers, which report up to 86% of oral mucosa yeast colonization in ionizing radiation treated patients (Belazi et al., 2004; Jham et al., 2007; Thaweboon et al., 2008). The data show that ionizing radiation actually interferes with the normal microbiota of the oral cavity, enhancing yeast colonization by two or threefold.

In the past, *C. albicans* was the species most frequently isolated from HNTP. More recently, however, an increased incidence of NAC species has been observed in

these patients (Kurtzman & Fell, 1998). Among *Candida*-colonized subjects in the present study, a larger number of *Candida* species was found in the patients group than in the control group (6 vs 2), as well as a higher frequency of patients colonized by NAC (64.7% vs 12.5%). These data show that ionizing radiation not only enhances the frequency of *Candida* colonization, but also enables colonization by species that are normally absent from healthy hosts.

Regarding yeast colonization during radiotherapy, 45% of the patients were already colonized by *C. albicans* and two NAC species (*C. glabrata* and *C. tropicalis*) before treatment started. However, *C. albicans* achieved the most constant colonization in these patients, being present before and during most of the radiation therapy in two patients.

NAC species have been described as emerging species, causing fungal infections in immunocompromised hosts (Redding, 2001; Redding et al., 2004). Accordingly, another 45.5% of the patients were colonized during radiation therapy by NAC species (*C. glabrata, C. tropicalis, C. parapsilosis*). Redding et al. (2002) reported the first three recorded cases of head and neck cancer patients under radiotherapy presenting oral mucosa infection by *C. glabrata*. It is interesting to note that *C. glabrata* was the NAC species that most persistently colonized the HNTP in the present study.

Some authors have reported an increased prevalence of NAC infections, such as *C. krusei, C. tropicalis* and *C. glabrata,* in immunocompromised patients, suggesting that this phenomenon is related to the use of the fluconazole (Belazi et al., 2004; Redding et al., 2004). Nevertheless, the diversification of *Candida* species found in the present study cannot be explained by resistance to antifungal drugs, as MIC values were similar between strains isolated from the patient and control groups, and also between strains isolated before and during treatment. These results were expected, since the patients had not been subjected to antifungal treatment.

Of the five patients colonized by *Candida* spp. before treatment began, two harbored *C. albicans* (patients 12 and 20). These samples were selected to assess the effect of radiotherapy on yeast virulence, as *C. albicans* is considered a serious pathogen for immunocompromised hosts (Redding et al., 2004).

Although genetic background and the physiological state of the host are considered the primary factors governing the etiology of *Candida* infection (Elahi et al., 2000), several fungal factors are crucial for its survival and pathogenesis (Schaller et al., 2002). One of the factors that can change the state of *C. albicans* from saprobic to pathogenic is its dimorphism, which allows invasion of the host tissue, causing infections. These morphogenic changes are influenced by the environmental conditions in which the fungus is growing (Gow, 1994).

As germ tube development is considered an important virulence factor, we measured its lenght and frequency throughout the radiotherapy. We found a significant increase in the average lenght of the *C. albicans* germ tubes in isolates from patients 12 and 20 as treatment progressed. We also found a tendency for the frequency of the germ tubes to rise during therapy, though it was statistically significant only in isolates from patient 12. Paula et al. (1990) also showed that radiotherapy induces the development of filamentation in *C. albicans* cultured from the HNTP. The increase of the frequency and lenght of the *C. albicans* germ tubes may enhance yeast adhesion to mucosal cells, consequently improving its invasive capability and making it potentially more virulent to the host.

Another very important *C. albicans* virulence factor related to radiation treatment in these two patients was the increase of yeast resistance to killing by macrophage. By contrast, no change was seen in the phagocytic index or the polymorphism of *C. albicans* inside the macrophages during treatment. Consequently, the acquisition of macrophage killing resistance cannot be explained by these factors. Similarly, Marodi et al. (1991) showed that virulent *C. albicans* and less virulent *C. parapsilosis* differ in their macrophage killing resistance, but not in their susceptibility to phagocytosis. The same authors also showed that *C. albicans* and *C. parapsilosis* differ in their resistance to lytic mediators, raising the possibility that this killing resistance might be a consequence of the acquisition of resistance to macrophage lytic mediators. Alternatively, the resistance to killing may be a secondary effect of the capacity of some *C. albicans* strains to induce mouse peritoneal macrophage apoptosis and/or necrosis (Panagio et al., 2002).

In conclusion, the data presented here indicate that radiotherapy may be an important factor for *C. albicans* virulence development. Some authors have suggested a decrease in the natural immune response of the oral mucosa as a consequence of the secretory dysfunction induced by radiotherapy (Ramirez-Amador et al, 1997; Nakashima et al, 2005). It is possible that the decrease of this natural immunity induced by the ionizing radiation would allow persistent *C. albicans* colonization and, consequently, the selection of yeast strains with higher adhesion and invasive capability, as well as higher resistance to the macrophage killing activity.

If that is the case, HNT patients might be a source of horizontal transmission of potentially more pathogenic *C. albicans* strains, which in turn could be disseminated into the healthy population.

RESUMO

Efeito da radioterapia sobre a freqüência de Cândida spp. e sobre a virulência de C. albicans isoladas da cavidade oral de pacientes com câncer de cabeça e pescoço

O objetivo deste estudo foi avaliar a diversidade e a prevalência de *Cândida*, bem como a virulência de *Cândida albicans*, isoladas da cavidade bucal no decurso de tratamento por radiações ionizantes de pacientes acometidos por tumores de cabeça e pescoço (PTCP). Amostras de 21 pacientes e 24 controles foram analisadas. *C. albicans* isoladas de dois pacientes ao longo do tratamento radioterápico foram avaliadas para fatores de virulência. A radioterapia induziu um grande aumento da colonização de *Cândida* como um todo (81% vs 33%) e *Cândida* não albicans (CNA) em particular (52.4% vs 4.0%) em PTCP quando comparado com controles não irradiados. Cinco espécies diferentes de CNA foram encontradas nos pacientes: *C. glabrata, C. tropicalis, C. parapsilosis, C. krusei* and *C. kefir.* Por outro lado, a colonização por *C. albicans* nestes pacientes e controles foi similar (6/21, 28.6% vs 7/24, 29.2%, respectivamente).

Além disso, dos 11 pacientes que foram avaliados antes e durante o tratamento radioterápico, 5 pacientes (45,5%) foram colonizados antes do início da radioterapia e outros 5 (45,5%) durante o tratamento radioterápico. Destes últimos, todos foram colonizados apenas com espécies CNA. Observou-se, ainda, um aumento contínuo e significante da virulência de *C. albicans* com o progresso da radioterapia nos dois pacientes estudados. Conclui-se que o tratamento radioterápico é um importante fator de desenvolvimento de candidíase oral, incluindo candidíase por espécies não albicans, em pacientes portadores de tumor de cabeça e pescoço. A radioterapia pode, ainda, facilitar o desenvolvimento de cepas mais virulentas de *C. albicans*.

Palavras chave: *Cândida*. Radioterapia. Fator de virulência. Candidíase oral.

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