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Oral *Candida* isolates in patients undergoing radiotherapy for head and neck cancer: prevalence, azole susceptibility profiles and response to antifungal treatment

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Oral pseudomembranous candidiasis and mucositis were assessed in 39 patients receiving a total dose of 39–70 Gy radiotherapy for head and neck cancer. Mucositis was scored using the Radiation Therapy Oncology Group criteria, and oral candidiasis was diagnosed on the basis of clinical evaluation and quantitative laboratory findings. Radiation-induced mucositis was observed in 9/39 patients. Only 3/39 patients discontinued radiotherapy due to acute severe mucosal effects. Candidiasis (colony-forming units 35 to ≥60/lesion) associated with mucositis was diagnosed in 30/39 patients: the most frequent aetiology of the infection was *Candida albicans* (n = 23), followed by *Candida glabrata* (n = 3), *Candida krusei* (n = 2), *Candida tropicalis* (n = 1) and *Candida kefyr* (n = 1). Patients with confirmed oral pseudomembranous candidiasis were treated with either fluconazole 200 mg/day or itraconazole 200 mg/day for 2 weeks. Clinical improvement and concomitant negative *Candida* cultures (mycologic cure) were the criteria determining a response to antifungal treatment. Etest revealed very low voriconazole MICs (0.004–0.125 μg/ml) for all isolates, and fluconazole resistance for eight *C. albicans* strains (MIC > 64 μg/ml) and for the *C. krusei* isolates (MIC > 32 μg/ml). The same strains showed itraconazole susceptibility dose dependence (MIC 0.5 μg/ml). Despite the itraconazole susceptible dose dependent MIC readings, all patients with oral pseudomembranous candidiasis caused by these strains responded to antifungal treatment with 200 mg/day itraconazole. Oral mycologic surveillance of patients undergoing radiotherapy for head and neck malignancies and susceptibility testing of isolates may be indicated in cases with mucositis-associated confirmed oral pseudomembranous candidiasis to ensure prompt administration of targeted antifungal treatment. On the basis of the low MIC values found, clinical evaluation of voriconazole is indicated for management of oral pseudomembranous candidiasis refractory to other azoles.

Key words: antifungals; oral pseudomembranous candidiasis; radiotherapy; response to treatment

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Over the past few decades there has been a dramatic rise in the number of immunocompromised patients. Many factors have contributed to this increase, such as antineoplastic radio/chemotherapy, the increased frequency of solid organ and bone marrow transplantation, the growing number of patients requiring intensive care, and the increasing number of HIV/

AIDS patients (10, 23, 28, 29). These immunocompromised individuals are particularly susceptible to opportunistic fungal infections, such as candidiasis, cryptococcosis, and aspergillosis, which rarely cause disease in healthy subjects (1, 12, 15, 40). Although *Candida albicans* is a common colonizer of the oral mucosa and can cause mild to severe lesions in immunocompromised or critically ill patients, many other *Candida* species, such as *Candida glabrata, Candida tropicalis, Candida krusei, Candida parapsilosis, Candida kefyr*, and *Candida dubliniensis* have been found to be responsible for oral infections (37, 38).

In patients with head and neck tumors who are undergoing radio/chemotherapy, mucositis is a common inflammation of the oral mucosa and the most frequent debilitating complication of cytotoxic cancer therapy (11), as the oral cavity is highly susceptible to direct and indirect toxic effects of cancer chemotherapy and ionizing radiation. The main clinical sign of cytotoxic therapy is radiation-induced xerostomia. Moreover, oral epithelium, salivary glands, muscle, bone, and teeth can be directly affected (35, 36). Previous work has shown that after approximately 2 weeks of irradiation therapy, the number of *Candida* species is remarkably increased and oral candidiasis concurrent with mucositis adds to the patient's discomfort, often leading to discontinuation of treatment (6, 8). Moreover, high yeast levels can persist for at least 6 months following completion of radiotherapy (30).

The aims of the present study were to assess the occurrence of oral yeast infections, to determine the incidence of *Candida* species, and to evaluate whether the choice of azole therapy can be derived from susceptibility data. For this purpose a cohort of 39 patients undergoing radiotherapy for the management of head and neck cancer was studied. *In vitro* susceptibility of oral *Candida* isolates can be useful in selecting the appropriate treatment for the best therapeutic outcome in patients with confirmed infections.

Material and methods

Sample population

Thirty-nine randomly selected consecutive patients receiving radiation therapy for head and neck cancer, but not receiving cytoprotection or cytokine granulocyte macrophage colony-stimulating factor (GM-CSF) for controlling radiation induced mucositis, were examined in the Department of Oral Medicine and Maxillofacial Pathology, School of Dentistry, Aristotle University of Thessaloniki, Greece, during March 2003 and July 2003. The oral hygiene status, periodontal condition, presence of plaque, smoking and drinking habits were recorded. The patients were ebing treated for oral squamous cell, laryngeal, and oropharyngeal carcinoma (Table 1). The mean age of the study population, comprising 26 males and 13 females, was 56.8 years (range 39–73). All participants had received head and neck radiotherapy (total dose 39–70 Gy), in five weekly sessions (daily dose 1.8–2.0 Gy), in the State Hospital of Anticancer Therapy, Thessaloniki, Greece. They were all evaluated intraorally at the beginning of the study and at each weekly radiotherapy session. The periodontal condition, the presence of plaque, and the presence/absence of dentures were also recorded for each patient in the study. Mucositis was scored (Table 2) using to the Radiation Therapy Oncology Group criteria (31), and oral candidiasis was diagnosed on the basis of clinical assessment and on quantitative and qualitative laboratory findings. Depending on the severity of the condition, patients diagnosed with severe mucositis were managed with corticosteroid agents at a dose of 4 mg, three times a day. The study group was followed for 10 weeks, so as to record any recurrence of pseudomembranous candidiasis and to evaluate antifungal treatment regimens after completion of the 5-week session of radiotherapy. Informed consent was obtained from all patients.

Table 1. Clinical features of 39 patients with head and neck cancer

Type and anatomic site of tumor	No. of patients
Squamous cell carcinoma	
lower lip	7
tongue	7
buccal	4
oropharynx	6
glottic	2
supraglottic	6
hard palate	3
floor of the mouth	2
Osteosarcoma	
hard palate	2
Total	39

Mycology

Evaluation of the clinical signs and symptoms led to suspicion of oral candidiasis, which was confirmed after detection of the gram-positive yeast cells and/or hyphae at high density upon microscopic examination of tissue scrapings obtained with a sterile surgical blade from the oral lesions. A portion of tissue scrapings and material obtained from the oral lesions by rubbing with sterile nontoxic cotton swabs were used for cultures. Samples from all subjects were taken at the end of the second and third radiotherapy session and at the same time of each day (9 and 11 a.m.). Specimens from each patient were inoculated in Sabouraud dextrose agar (SDA) with 50 mg/ml chloramphenicol, and in CHROMagar Candida^{TM} (CHROMagar Microbiology, Paris, France), for isolation and presumptive identification of *Candida* species. The presence of yeast elements in tissue scrapings and the associated confluent yeast growth in colony forming units (CFUs) from each lesional specimen were the laboratory criteria for yeast infection. The identity of each isolate was corroborated with the API 32ID (BioMerieux, Marcy l'Etoil, France). All *C. albicans* isolates were screened for differentiation from *C. dubliniensis* with additional assays including observation of morphologic characteristics in 50 g staib agar (18) (50 g *Guizotia abyssinica* [sterile bird seed extract (17)], 1 g glucose [Sigma, St. Louis, MS, USA], 1 g KH_2PO_4, 1 g creatinine and 15 g agar

Table 2. Patients with radiation-induced mucositis and mucositis associated with candidiasis between the second and fifth week of radiotherapy

Grades/phase of mucositis	No. of patients with mucositis	No. of patients with mucositis and oral pseudomembranous candidiasis			Total No. of patients
	2nd week	3rd week	4th week	5th week	
I – Inflammatory/vascular phase	4	0	0	0	4
II – Epithelial phase	5	7	5	0	17
III – Ulcerative/bacteriological	0	6	4	2	12
IV – Healing phase	0	2	2	2	6
Total	9	15	11	4	39

[both from Sigma, St. Louis, MS, USA]), 1000 ml distilled water, indirect immunofluorescence (4), and polymerase chain reaction (PCR). The minimum inhibitory concentrations (MICs) of the species to fluconazole and itraconazole, as well as to the newer azole voriconazole, was determined separately on five single CFUs from each isolated species with Etest® (AB Biodisk, Solna Sweden), following the manufacturer's instructions and using *C. parapsilosis* ATCC 22019 and *C. krusei* ATCC 6258 as quality control (QC) strains (21, 38, 39). Five colonies from each quality control strain were also tested by the broth microdilution method to verify that the MIC values were within the recommended NCCLS M27-A2 24-h MIC limits (21), and in agreement with the respective Etest MIC limits. A median MIC value was calculated for each set of five colonies tested and subsequently an MIC range was established for each *Candida* isolate and quality control strain challenged against the azoles tested. As the azole BMD and Etest MICs for the QC strains were within the expected range (25–27), no broth microdilution (BMD) was deemed necessary for the clinical isolates.

Patients with clinically and laboratory-confirmed oral yeast infections were treated with either fluconazole 200 mg/day or itraconazole 200 mg/day for 2 weeks. The choice of drug was based on MIC findings. Patients with pseudomembranous candidiasis attributed to fluconazole-susceptible isolates were treated with fluconazole and those with fluconazole-resistant isolates with itraconazole. Clinical improvement and concomitant negative *Candida* cultures (mycologic cure) were the criteria for response to antifungal treatment.

Results

All 39 patients receiving radiotherapy for head and neck tumors showed a significant increase in manifestations of oral inflammation and lesions in the second and third week of radiation therapy. Patient symptomatology included sore mouth, lip dryness and xerostomia, taste alteration, ulcers, pain, dysphagia, disallowing, and inability to talk. Oral mucositis, without candidiasis, was diagnosed after the second week of radiotherapy in 9/39 (23%) patients (Table 2). Management of these patients included the use of topical factors for alleviation of discomfort, such as xylocaine cream, oral ice chips, chlorhexidine gluconate mouthrinse 0.12%, and pilocarpine (salagen).

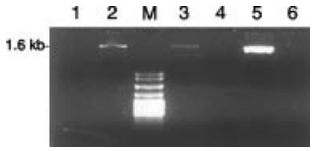

Fig. 1. Screening *C. albicans* isolates for *C. dubliniensis* by PCR. All 23 *C. albicans* isolates tested yielded the expected 1.6-kb product (17), as seen in Lanes 2, 3, and 5. The positive control Type strain *C. dubliniensis* NCPF 3949 template DNA used yielded no amplification product (Lanes 1, 4, and 6). M: Molecular size marker, *Msp* I digest of pBR322 DNA.

C. albicans was the predominant species isolated from 23/39 (59%) patients. Conversely, *C. dubliniensis* was not identified in any of the isolates (Fig. 1). Other non-*C. albicans* species identified were: *C. glabrata* in 3/39 patients (8%), followed by *C. kefyr* in 2/39 patients (5%), *C. tropicalis* in 1/39 patients (3%) and *C. krusei* in 1/39 patients (3%). All specimens from lesions with microscopically confirmed invasion produced confluent yeast growth ranging from 35 to >60 CFU. Oral mucositis without candidiasis was diagnosed in 9/39 patients (23%) (Table 2). Mild periodontal disease (without gingival bleeding and pocket depth) was observed in two of these patients. None of these patients had a history of smoking or alcoholic drink consumption. Management of two patients with oral mucositis grade II and mild periodontal disease included use of corticosteroid agents 2 mg, twice daily. Topical treatments for alleviation of discomfort, such as xylocaine cream, oral ice chips, chlorhexidine gluconate mouthrinse 0.12%, and pilocarpine hydrochloride, were administered to all patients with mucositis.

Oral mucositis grades II–IV with pseudomembranous candidiasis was diagnosed in 30/39 patients (77%) (Table 3). The status of oral hygiene of these patients was good, with no periodontal disease or dental plaque observed. The severity of mucositis or pseudomembranous candidiasis was not associated with smoking or alcohol consumption, as only 5/30 patients had a history of smoking (20 cigarettes/day) and none was an habitual consumer of alcohol. The majority of our patients (36/39) completed the course of radiotherapy. Three patients discontinued radiotherapy due to acute severe mucosal effects, which were managed with corticosteroids (Medrol, 4 mg/three times daily); radiation treatment was then resumed and completed. After completion of radiotherapy, five (5/30) patients presented with oral candidiasis due to *in vitro* fluconazole refractory *C. albicans* strains and received itraconazole (200 mg/day) for 15 days before the infection was cleared.

Susceptibility testing of isolates showed that 8/23 *C. albicans*, 2/3 *C. glabrata,* and 2/2 *C. krusei* isolates were resistant to fluconazole and susceptible dose dependent to itraconazole, whereas all isolates demonstrated very low voriconazole MICs (Table 3). The broth microdilution (BMD) and Etest 24-h MIC range for the quality control strains *C. parapsilosis* ATCC 22019 and *C. krusei* ATCC 6258 were within the recommended range at every test occasion (21). Specifically, the recorded quality control BMD MIC range of fluconazole, itraconazole, and voriconazole for *C. parapsilosis* ATCC 22019 was 0.25–2 μg/ml, 0.125–0.5 μg/ml, and 0.025–0.25 μg/ml, respectiviely. For *C. krusei* ATCC 6258 the BMD MIC range was

Table 3. Candida species from 30 patients with mucositis associated with oral candidiasis and Etest minimum inhibitory concentration (MIC) median range of three azole antifungals

Candida isolates	No. of strains	No. of *Candida* CFU isolated	Median range of antifungal agent MIC_{50} (MIC μg/ml)		
			Fluconazole	Itraconazole	Voriconazole
C. albicans	23	40–>60	1–>64[a]	0.016–0.5[a]	0.004–0.125
C. glabrata	3	40– >60	4– >32[b]	0.125–0.5[b]	0.064–0.125
C. krusei	2	35–>60	>32	0.023–0.25	0.094–0.025
C.tropicalis	1	>60	4	0.047	0.047
C. kefyr	1	>60	1	0.016	0.023
Quality control strains					
C. parapsilosis ATCC 22019			0.25–2	0.125–0.5	0.025–0.25
C. krusei ATCC 6258			32–>64	0.06–0.5	0.047–0.25

The MIC interpretive criteria of fluconazole and itraconazole were adopted from the National Committee for Clinical Laboratory Standards (NCCLS) guidelines (21) M27-A2 (2002). No NCCLS interpretive criteria are set for voriconazole.
[a]The same eight strains.
[b]The same two strains.

32–64 μg/ml, 0.06–0.5 μg/ml, and 0.047–0.25 μg/ml, respectively. Persistent hazy growth (trailing phenomenon) of some *C. albicans* isolates and of the *C. tropicalis* strain was observed around the Etest strip inhibition ellipse of fluconazole and itraconazole. In such cases, the MIC was read ignoring the hazy (discernible) growth (25–27).

Discussion

Mucositis is recognized as the leading complication during radiotherapy for head and neck tumors (10, 19, 40). Considered a multifactorial biological process, the severity of mucositis is related to the dose rate and total dose of therapy as well as to the presence of local irritation, secondary infection, and xerostomia (13, 14, 32). Irradiation-induced oral mucositis is classified as a reactive inflammatory process due to tissue reaction in which the oral microflora may also play a role in progressive mucosal damage. Furthermore, poor oral hygiene is often associated with more severe mucositis, where the sensitive mucosal tissues can easily become infected by microorganisms, including fungi, herpes viruses and bacteria (20, 35). However, the patients in this study did not present with poor oral hygiene, dental plaque or severe periodontal disease. Therefore, the recorded grades of mucositis and the concomitant yeast infections were not associated with the oral condition of the patients or with their smoking and alcoholic consumption habits, as a minority of our patients had a history of smoking and none was a moderate or heavy drinker.

Oral mucositis grades II–IV with oropharyngeal candidiasis were manifested in 30/39 patients (Table 3). These findings are in accordance with reports of a high percentage (90%) of *Candida* infection in cancer patients undergoing radiotherapy (8, 10, 22, 29) and oral mucositis and fungal infections of 40–70% during antineoplastic therapy (3, 13, 33).

The recurrence of pseudomembranous candidiasis after completion of radiotherapy was attributed to postradiotherapy oral yeast colonization with concomitant clinical infection (7, 29, 30). In accordance with previous observations (24, 29), *C. albicans* was the predominant species (77%), isolated from lesions of 23/30 patients, whereas 23% of the isolates were non-*C. albicans* species (Table 2). Screening *C. albicans* isolates for the presence of *C. dubliniensis* was negative (Fig. 1). Unlike previous observations of patients undergoing radiotherapy (22), no multiple species were isolated from the oral lesions of our patients. All specimens from lesions with microscopically confirmed invasion produced confluent yeast growth. Yet, tissue invasion by strains of *Candida* species such as *C. albicans*, *C. glabrata* and *C. krusei* was seen even when the number of CFUs was as low as 35–40. This could be attributed to the ability of these *Candida* species to invade tissues in the early stages of the infection, when the number of CFUs is low. However, given the small number of observations in this study, the role of a random sampling factual error in the occurrence of this phenomenon cannot be excluded.

In the group of patients with mucositis and xerostomia, without signs and symptoms of candidiasis, *C. albicans* was found colonizing the oral mucosa of 5/9 patients (yeast CFU < 10). This was attributed to the reduction of saliva, predisposing patients to yeast colonization. Temporary xerostomia without *Candida* colonization was recorded in 4/9 patients. Varying degrees of temporary or permanent xerostomia without pseudomembranous candidiasis have been recorded before (9, 33), due to radiotherapy-induced injury of the salivary glands and the resulting atrophy of the secretor components.

Interestingly, the same *C. albicans* and *C. glabrata* strains that were *in vitro* resistant to fluconazole were susceptible dose dependent to itraconazole. Despite the susceptible dose dependent MIC readings (21) for itraconazole (0.125–0.5 μg/ml), all patients with mucosal lesions caused by such strains responded to 15 days' itraconazole treatment (200 mg/day). A similar itraconazole therapeutic outcome has been noted before in a cancer patient with *Candida lusitaniae* vaginal infection (16). Correlation of *in vitro* susceptibility test results and *in vivo* outcome has been substantiated using a murine model of invasive candidiasis in relation to fluconazole treatment (2). Therefore, the response to itraconazole treatment observed in our group of patients could indicate that itraconazole susceptible dose dependent *Candida* isolates express similar molecular mechanisms as fluconazole susceptible dose dependent isolates (24, 34).

This study supports the requirement for regular stomatologic and mycologic surveillance of patients undergoing radiation therapy for head and neck malignancies. *In vitro* susceptibility testing of isolates may be indicated in cases with confirmed candidiasis, as is the case with *Candida* opportunistic infections affecting other high-risk groups of patients (5). Such testing can detect resistance to the azoles routinely used for management of the infection and can encourage prompt administration of the targeted antifungal treatment that is often a prerequisite for the unremitting completion radiotherapy. In such cases, voriconazole can be an alternative regimen for managing oral candidiasis.

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