Journal of Radiotherapy in Practice

Journal of Radiotherapy in Practice (2013)
12, 100–104

doi:10.1017/S1460396911000446

Original Article

Oral candidiasis: Species identification and their antifungal susceptibility pattern in cancer patients receiving radiation therapy

Pratibha M. Mane, Y. Hyma Pratyusha

Department of Microbiology, Government Medical College, Aurangabad, Maharashtra, India

(Received 18 August 2011; revised 17 October 2011; accepted 17 October 2011)

Abstract

Introduction: Radiotherapy-induced hyposalivation encourages oral *Candida* colonization that often leads to oral/pharyngeal candidiasis. The objective of this study was to identify the *Candida* species in lesions of oropharyngeal candidiasis in patients undergoing radiotherapy for head and neck cancers and to find out antifungal susceptibility pattern.

Material and methods: Swabs were collected from 60 patients who developed lesions suggestive of oral candidiasis at the end of 1st week of radiation therapy. Antifungal susceptibility of each of the isolated species was done using disc diffusion method following CLSI guidelines.

Results: Candida was isolated in 13 cases. *C. albicans* (7) was the most predominant species; a small number of other species have also been identified. Few strains (3) of *Candida* showed variable resistance to the commonly used antifungal drugs.

Discussion: The colonization of *Candida* may lead to development of infections with drug-resistant strains, and hence the patients receiving radiation for head and neck cancers should undergo microbiological study for oral candidiasis.

Keywords

Candidiasis; radiotherapy; antifungal drugs

INTRODUCTION

Role of chemotherapy and radiotherapy has increased enormously in effective management of various types of cancer in recent past. This success story has other side also and includes the severe form of side effects of these therapies. The important being the disruption in the functions and integrity of the mouth.[1] *Candida albicans* is a normal inhabitant and frequently colonizes in the oral cavity in a large population of normal individuals and does not cause disease under normal circumstances. The changes in systemic as well as local factors may lead to clinical oral fungal infections. The factors responsible for oral fungal infection include immunosupression, changes in normal oral

Correspondence to: Dr Pratibha M. Mane, MD, Assistant Professor of Microbiology, Mamata Medical College, Khammam, A.P. India.
E-mail: drsachinkolte@sify.com

microbial flora and local tissue damage. Cancer patients receiving radiation or chemotherapy are prone for all these risk factors.[2–3] Radiotherapy-induced hyposalivation encourages oral *Candida* colonization that often leads to oral and/or pharyngeal candidiasis. Compromised salivary function secondary to destruction of glandular tissue by radiation is thought to be a major factor leading to *Candida* infection.[4]

Oral candidiasis can present as various clinical presentations, such as pseudomembranous candidiasis (thrush), chronic hyperplastic candidiasis, erythematous candidiasis, and angular cheilitis. Whatever the presentation, oral candidiasis have a significant impact on quality of life and can impair nutritional status. As oral candidiasis cause burning sensation while eating and impairs significantly taste sensations, it may adversely affect the food intake by the patients. In patients suffering with cancer, maintenance of nutritional intake is very important.

If neglected, the colonized *Candida* can spread to other organs and may result in systemic effects or septicemia. Identification of oral candidiasis forms an important part of management of cancer patients. Determination of the epidemiology of *Candida* isolates in patients receiving head and neck radiation has traditionally involved taking individual cultures and identifying yeasts to the species level, as well as using techniques to identify specific yeast strains with serotyping and biotyping. In the past, *Candida albicans* has been by far the most predominant organism isolated.[5]

Recently published studies have shown that 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.[2,6]

Fluconazole is the predominant medication used to treat oropharyngeal candidiasis caused by *Candida albicans*.[5] Some studies have reported an increase in *Candida* resistance to first-line antifungal agents such as fluconazole. Hence early recognition of oral candidiasis and the subsequent speciation and susceptibility testing is important.[3]

Oral candidiasis can be easily treated particularly in earlier stages, so it is important to identify and treat or take preventive measures in the high-risk cancer patients.[3]

The objective of this study was to identify the *Candida* species causing oropharyngeal candidiasis in patients suffering with oral cavity cancers undergoing radiotherapy and to find out antifungal susceptibility pattern.

MATERIAL AND METHODS

Patients undergoing radiation therapy for oral cavity cancers were included in this study.

Informed and written consent was taken from each patient before the sample collection. The ethics committee of Government Medical College, Aurangabad gave approval for this study. The study was carried over a period of 1 year in the Department of Microbiology, Government Medical College and Hospital, Aurangabad, Maharashtra, India. This is a tertiary care hospital and training centre providing facility of radiotherapy and/or chemotherapy to the patients suffering from cancers.

Swabs were collected from 60 patients who developed lesions suggestive of oral candidiasis such as erythematous, hyperplastic, or pseudomembranous patches. At the end of 1st week of radiation therapy, two swabs were collected from each patient with sterile cotton swabs. One swab was used for Gram staining, and the other was used for culture on Sabouraud's dextrose agar.

In the case of positive culture, yeast identification was done by conventional methods like germ tube test and corn meal agar was used to study detail morphological features such as presence of chlamydospores, pseudohyphae, true mycelium and blastospore arrangement. For further characterization, each isolate was subjected to carbohydrate assimilation and fermentation tests. To differentiate *Candida*

https://doi.org/10.1017/S1460396911000446 Published online by Cambridge University Press

albicans from *C. dubliniensis*, all *C. albicans* isolates were subjected to growth at 45°C. Infection was defined in case of growth on culture media and clinical signs of oral candidiasis. Antifungal susceptibility of each of the isolated species was done using disc diffusion method following CLSI guidelines. Antifungal discs (HiMedia Laboratories Limited) used were Nystatin (100 units/disc), Amphotericin B (100 units/disc), Fluconazole (10 μgm/disc), Clotrimazole (10 μgm/disc). ATCC *C.kefyr*-8614 was used as a control strain.

RESULTS

Majority of patients were men in the age group of 51–70 years. On Gram stain, 8 swabs showed presence of yeast cells and pseudohyphae and 13 swabs showed growth on Sabouraud's dextrose agar. Out of 13 isolates, 7 were Germ tube test positive.

The species isolated are shown in Table 1.

DISCUSSION

Cancer and cancer treatment profoundly impairs oral health. Oral complications presenting as mucositis, xerostomia, bleeding and infections are commonly seen. The clinical diagnosis of oral infections may be difficult due to reduced inflammatory responses in the immunocompromised host. It has been found that oropharyngeal colonization of *Candida* species may increase the risk of systemic infection especially when oral ulcers develop during the neutropenic episodes. Risk factors for systemic fungal infection also include the use of broad-spectrum antibiotics and steroids.[7]

Table 1. *Table 1 shows Candida species isolated*

Organism	No.	Percentage
Candida albicans	7	54.2
Candida tropicalis	3	23.0
Candida krusei	1	7.6
Candida glabrata	1	7.6
Candida parapsilosis	1	7.6
Total	13	100

Chemotherapy or radiotherapy induced local tissue damage leads to oral mucositis. Damage to salivary glands results in hypofunctioning of salivary glands ultimately compromising the oral hygiene badly.[2] These factors probably play a major role in causation of oral candidiasis. The epidemiology of *C. albicans* and other yeasts from the oropharynx of patients receiving radiation for head and neck cancer is quite varied. *C. albicans* is the predominant organism associated with symptomatic infection.[5] In recent studies, *C. glabrata* has emerged as a notable pathogenic agent in oropharyngeal candidiasis in patients receiving radiotherapy.[8]

The incidence of colonization and infection of *Candida* has been reported from 43–90% and 13–52%, respectively.[4–6,9–10] In our study, we found the fungal infection in 21.6% of cases, corresponding with the other studies.

Still *Candida albicans* is the dominant species causing the oropharyngeal candidiasis in immunocompromised patients, dental wearers, systemic malignancies or patients undergoing radiation therapy. The emergence of other species can be attributed to prophylactic use of antifungal medications.[3–8,11–15]

The identification of species causing candidiasis is important and crucial for both clinical treatment and epidemiological studies. As pathogenicity and antifungal susceptibility varies among various species, an essential prerequisite for the laboratories is a suitable culture medium which facilitates the recovery and differentiation of *Candida* species. The conventional identification of pathogenic fungi in the clinical microbiology laboratory involves the examination of colony morphology, microscopic morphology and the assessment of various biochemical reactions. The presence of more than one *Candida* species in the oral cavity of the same host is not uncommon. By conventional methods, identification of yeasts may take several days, and, in presence of mixed cultures, it is difficult to isolate and identify the organisms. For differentiation of respective species of *Candida*, various phenotypic methods have been reported such as formation of chlamydospores; the pattern of carbohydrate assimilation, D-glucosidase activity, Staib agar, Niger agar, Tobacco

https://doi.org/10.1017/S1460396911000446 Published online by Cambridge University Press

Table 2. *Table 2 shows antifungal susceptibility pattern.*

Antifungal agents	Sensitive	Intermediate	Resistant	No. of isolates	Percentage (Resistant strain)
Nystatin	11	1 (*Candida albicans)*	1 (*Candida albicans)*	13	7.7
Amphotericin B	13	0	0	13	0.0
Clotrimazole	12	1 (*Candida albicans)*	0	13	0.0
Fluconazole	10	0	3 (*Candida albicans* [2] and *Candida tropicalis* [1])	13	23.0

agar and growth in Sabouraud agar at temperatures between 42 and 45°C.[16] There are new CHROM medias which are being advocated in identifying the *Candida* species. New chromogenic media can complement traditional identification methods for identifying clinical yeast isolates.[17–18] Recently, DNA-based methods such as DNA–DNA reassociation, DNA fingerprinting and southern hybridization with appropriate DNA probes have been described to identify *Candida* species in culture and in clinical materials. Currently, there are other molecular techniques apart from DNA-based methods such as pulsed field electrophoresis and PCR-based methods.[16] However, these genotypic methods have the disadvantage of being laborious and time-consuming, and besides they require specialized equipments.[19–20] These state-of-the-art techniques are not readily available to the majority of laboratories in India. Hence, identification of organisms largely rests on traditional culture and biochemical studies in Indian setup.

Chakrabarti et al.[21] expressed that the various methods done for detection of antifungal susceptibility pattern are having very desperate outcomes, and the results are often noncomparable. The disc diffusion method for study of antifungal susceptibility pattern is simple and easy to perform. Reliable, reproducible antifungal susceptibility tests depends on standardization of several factors like pH, inoculum size, liquid versus solid medium, medium formulation, time and temperature of inoculation. They observed that 2–5% *Candida* strains were resistant to Amphotericin B and 10–22% strains were intermediate. In case of Fluconazole, resistance was observed in 13–20% strains. These simple disc diffusion methods can suitably be standardized in the laboratories as it showed a good correlation with the reproducible broth dilution method. However, when the results are equivocal, especially for azoles, the broth dilution test should be used.[21]

In our study, few strains of *Candida* showed resistance to the drugs used; (Table 2) however, the number studied is too low to make any decisive comment. Topical agents are considered preferable to systemic agents due to lower risk of side effects and drug interactions. The previously published studies have used fluconazole as tropical agent, and it had shown to be very effective in the prevention of clinical oral fungal infection and in reducing oral fungal colonization in patients receiving cancer therapy. Lately, studies are being published showing resistance to Fluconazole[3] as in our study. A close monitoring is required for frequency of different non-*albicans Candida* species isolated from patients with systemic infection and of antifungal susceptibility pattern because of recent increased use of Fluconazole as prophylactic agent against oral candidiasis.

The pathogenic agents colonizing the oral cavity may induce symptomatic infections, often systemic ones, and may promote the development of infections with drug-resistant strains. Regular dental check-up examinations, oral microbial surveillance and application of professional oral hygienic measures in patients with oral cancer may decrease the incidence, duration and severity of infectious complications. Considering the high prevalence of oral candidiasis in cancer patients, identification of more effective topical antifungal agents to avoid the potential side effects of systemic agents would be beneficial.

https://doi.org/10.1017/S1460396911000446 Published online by Cambridge University Press

LIMITATIONS OF THE STUDY

The number of positive culture is very small to make confirmatory remarks about the antifungal susceptibility pattern of the *Candida* species.

References

1. Worthington HV, Clarkson JE, Eden OB. Interventions for preventing oral mucositis for patients with cancer receiving treatment (Review). Interventions for preventing oral candidiasis for patients with cancer receiving treatment (review). Cochrane Database Syst Rev 2007; 1:CD003807.
2. Lalla RV, Latortue MC, Hong CH, Ariyawardana A, D'Amato-Palumbo S, Fischer DJ, Martof A, Nicolatou-Galitis O, Patton LL, Elting LS, Spijkervet FK, Brennan MT; Fungal Infections Section, Oral Care Study Group, Multinational Association of Supportive Care in Cancer (MASCC)/International Society of Oral Oncology (ISOO). A systematic review of oral fungal infections in patients receiving cancer therapy. Support Care Cancer 2010; 18:985–992.
3. Schelenz S, Abdallah S, Gray G, Stubbings H, Gow I, Baker P, Hunter PR. Epidemiology of oral yeast colonization and infection in patients with hematological malignancies, head neck and solid tumors. J Oral Pathol Med 2011; 40:83–89.
4. Koc M, Aktas E. Prophylactic treatment of mycotic mucositis in radiotherapy of patients with head and neck cancers. Jpn J Clin Oncol 2003; 33:57–60.
5. Redding SW, Zellars RC, Kirkpatrick WR, McAtee RK, Caceres MA, Fothergill AW, Lopez-Ribot JL, Bailey CW, Rinaldi MG, Patterson TF. Epidemiology of oropharyngeal Candida colonization and infection in patients receiving radiation for head and neck cancer. J Clin Microbiol 1999; 37:3896–3900.
6. Belazi M, Velegraki A, Koussidou-Eremondi T, Andreadis D, Hini S, Arsenis G, Eliopoulou C, Destouni E, Antoniades D. Oral Candida isolates in patients undergoing radiotherapy for head and neck cancer: prevalence, azole susceptibility profiles and response to antifungal treatment. Oral Microbiol Immunol 2004; 19:347–351.
7. Alberth M, Majoros L, Kovalecz G, Borbás E, Szegedi I, J, Márton I, Kiss C. Significance of oral Candida infections in children with cancer. Pathol Oncol Res 2006; 12:237–241.
8. Dongari-Bagtzoglou A, Dwivedi P, Ioannidou E, Shaqman M, Hull D, Burleson J. Oral Candida infection and colonization in solid organ transplant recipients. Oral Microbiol Immunol 2009; 24:249–254.
9. Nicolatou-Galitis O, Velegraki A, Sotiropoulou-Lontou A, Dardoufas K, Kouloulias V, Kyprianou K, Kolitsi G, Skarleas C, Pissakas G, Papanicolaou VS, Kouvaris J. Effect of fluconazole antifungal prophylaxis on oral mucositis in head and neck cancer patients receiving radiotherapy. Support Care Cancer 2006; 14:44–51.
10. Epstein JB, Freilich MM, Le ND. Risk factors for oropharyngeal candidiasis in patients who receive radiation therapy for malignant conditions of the head and neck. Oral Surg Oral Med Oral Pathol 1993; 76:169–174.
11. Jain PA, Veerabhadrudu K, Kulkarni RD, Ajantha GS, Shubhada C, Amruthkishan U. Comparative study of adherence of oral Candida albicans isolates from HIV sero-positive individuals and HIV sero-negative individuals to human buccal epithelial cells. Indian J Pathol Microbiol 2010; 53:513–517.
12. Lattif AA, Banerjee U, Prasad R, Biswas A, Wig N, Sharma N, Haque A, Gupta N, Baquer NZ, Mukhopadhyay G. Susceptibility pattern and molecular type of species-specific Candida in oropharyngeal lesions of Indian human immunodeficiency virus-positive patients. J Clin Microbiol 2004; 42:1260–1262.
13. González Gravina H, González de Morán E, Zambrano O, Lozano Chourio M, Rodríguez de Valero S, Robertis S, Mesa L. Oral Candidiasis in children and adolescents with cancer. Identification of Candida spp. Med Oral Patol Oral Cir Bucal 2007; 12:E419–E423.
14. Sebti A, Kiehn TE, Perlin D, Chaturvedi V, Wong M, Doney A, Park S, Sepkowitz KA. Candida dubliniensis at a cancer center. Clin Infect Dis 2001; 32:1034–1038.
15. Baradkar VP, Kumar S. Species identification of Candida isolates obtained from oral lesions of HIV infected patients. Indian J Dermatol 2009; 54:385–386.
16. Chavasco JK, Paula CR, Hirata MH, Aleva NA, Melo CE, Gambale W, Ruiz Lda S, Franco MC. Molecular identification of Candida dubliniensis isolated from oral lesions of HIV-positive and HIV-negative patients in São Paulo, Brazil. Rev Inst Med Trop Sao Paulo 2006; 48:21–26.
17. Eraso E, Moragues MD, Villar-Vidal M, Sahand IH, González-Gómez N, Pontón J, Quindós G. Evaluation of the new chromogenic medium Candida ID 2 for isolation and identification of Candida albicans and other medically important Candida species. J Clin Microbiol 2006; 44:3340–3345.
18. Abraham CM. Advances and emerging techniques in the identification, diagnosis and treatment of Oral Candidiasis. The Open Pathol J 2011; 5:8–12. DOI: 10.2174/1874375701105010008.
19. Liguori G, Lucariello A, Colella G, De Luca A, Marinelli P. Rapid identification of Candida species in oral rinse solutions by PCR. J Clin Pathol 2007; 60:1035–1039.
20. Das S, Brown TM, Kellar KL, Holloway BP, Morrison CJ. DNA probes for the rapid identification of medically important Candida species using a multianalyte profiling system. FEMS Immunol Med Microbiol 2006; 46:244–250.
21. Chakrabarti A, Ghosh A, Kanta A, Kumar P. *In vitro* antifungal susceptibility of Candida. Indian J Med Res 1995; 102:13–19.