International Journal of Research in Medical Sciences
Yogitha PPV et al. Int J Res Med Sci. 2015 May;3(5):1189-1194
www.msjonline.org
pISSN 2320-6071 | eISSN 2320-6012
DOI: 10.5455/2320-6012.ijrms20150529

Research Article

Isolation and speciation of genus candida in patients undergoing chemotherapy and radiotherapy for head and neck tumours

P. V. V. Yogitha, N. Lakshmi*, Koripella Rama Lakshmi, Perala Bala Murali Krishna, Sulakshana Sony Cheemala

Department of Microbiology, Andhra Medical College, Visakhapatnam, Andhra Pradesh, India

Received: 11 March 2015
Revised: 20 March 2015
Accepted: 09 April 2015

***Correspondence:**
Dr. N. Lakshmi,
E-mail: lakshmimicroamc@gmail.com

ABSTRACT

Background: Fungal infections occur as a result of defect in the immune system. The use of wide-spectrum antibiotics, immunosuppressive therapy, HIV and an increasing incidence of diabetes are some of the causes that resulted in raising number of immunocompromised individuals, in the global scenario. Opportunistic fungal infections mainly oral candidiasis is common in patients undergoing chemotherapy (CT) or radiotherapy (RT) for Head & Neck tumors. Objectives: Isolation and speciation of Candida with antifungal susceptibility testing in patients undergoing CT and RT for Head and Neck tumors.
Methods: Study group comprised of 100 saliva samples collected by oral rinse method, (50 chemotherapy and 50 radiotherapy) from inpatients of King George hospital, Visakhapatnam and 50 normal healthy individuals were taken as control group. Standard mycological tests for the Candida isolation, speciation and antifungal susceptibility were done.
Results: In the study group out of the 100 patients, 38% were culture positive for Candida. The most commonly isolated species was C. albicans (60.5%) followed by non albicans species. Most of the candida species showed sensitivity to nystatin, amphotericin B, itraconazole and Ketoconazole. In control group 9 out of 50 samples (18%) were culture positive and all the isolates were Candida albicans.
Conclusion: Increase in frequency of oral candidiasis in patients undergoing chemotherapy and radiotherapy was observed. The increase in positivity may be attributed to inadequate nutritional status and poor oral hygiene during chemotherapy and radiotherapy. Culture positivity is more in RT patients than in CT patients. Though C. albicans is the predominant isolate, non albicans species are also emerging. All the Candida species isolated from study group were sensitive to nystatin, amphotericin B, itraconazole and ketoconazole. In the study group all the Candida species were resistant to clotrimazole and fluconazole and in the control group all the C. albicans were sensitive to fluconazole.

Keywords: Candida albicans, Oral candidiasis, Radiotherapy, Fluconazole, Disk diffusion method

INTRODUCTION

Fungal infections occur as a result of defect in the immune system. The use of wide-spectrum antibiotics, immunosuppressive therapy, HIV and an increasing incidence of diabetes are some of the causes that resulted in raising number of immunocompromised individuals.

Opportunistic fungal infections mainly oral candidiasis is common in patients who undergo chemotherapy or radiotherapy. Radiation was given during treatment of

malignancy causes alteration of the oral environment, predisposing to the colonization of the oral mucosa by yeast species most frequently Candida.

Candida species are usually normal oral commensals and their transition to opportunistic pathogens may be associated with the virulence attributes of the organism and also the host resistance.[1] The virulence of Candida strain isolated from patients on chemotherapy and irradiation is largely determined by activities of adherence, multiplication and release of cytoplasmic antigen, enolase and Candida aspartic proteinase.[2]

Although Candida albicans remains the most common causative agent of both superficial and deep fungal infections, an increased incidence of the non albicans species like C. tropicalis, C. krusei and C. parapsilosis were also observed.[3]

Objectives

1) To study the prevalence of Candida in patients undergoing chemotherapy and radiotherapy for head and neck tumors.

2) Isolation and speciation of Candida in those patients.

3) Antifungal susceptibility test for Candida species by Disk diffusion method.

METHODS

The present study was carried out in the dept. of microbiology, Andhra medical college, Visakhapatnam, India. Study group comprised of 50 chemotherapy and 50 radiotherapy inpatients of King George hospital, Visakhapatnam and 50 normal healthy individuals were taken as control group. The patients with other risk factors for candidiasis such as diabetes, recent usage of corticosteroids or antibiotics as well as patients using intraoral prostheses and patients who had received antifungal therapy were excluded from the study.

The samples were collected from control group and study group as follows:

50 patients whose age and sex were matched with study groups and apparently healthy, with no systemic diseases after routine investigations were taken as controls.

50 patients, who were on chemotherapy and 50 patients who were on radiotherapy for head and neck tumours of age 20-80 years were taken as study group.

After an informed consent, saliva samples were collected after oral rinse with phosphate buffer saline for 1 minute in a sterile plastic container. Direct microscopic examination of Gram stained smear (Figure 1) and KOH wet mount (Figure 2) of the sample was done and examined for budding yeast cells and pseudohyphae.

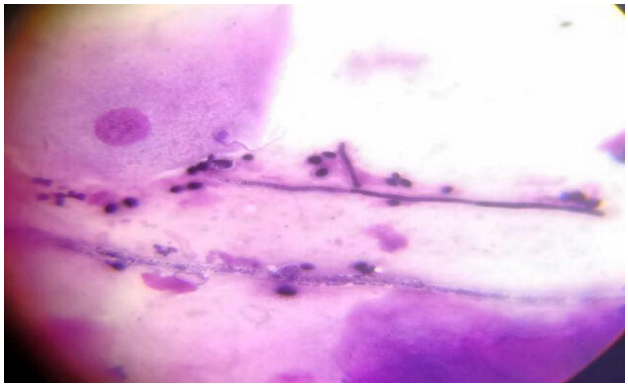

Figure 1: Gram stained smear showing budding yeast cells.

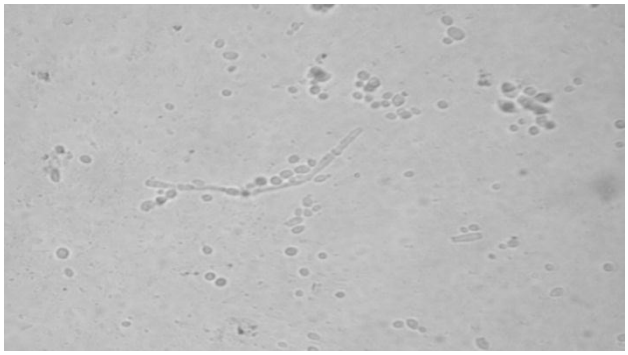

Figure 2: KOH mount showing pseudohyphae and budding yeast cells.

Samples were inoculated on Sabouraud's dextrose agar with gentamycin and incubated at 25°C and 37°C for up to 48-72 hours. Colonies appeared within 1-3 days as creamy white, smooth, pasty with a yeasty odour (Figure 3). Standard mycological tests were used to identify the isolates.[4]

Figure 3: Growth on SDA.

Candida isolates were identified by microscopic examination of Gram stained smear (Figure 4), germ tube

formation, chlamydospore and blastoconidia production which was tested on Cornmeal agar by Dalmau culture plate technique. Rapid method of identifying C. albicans is by its ability to form germ tubes within two hours when incubated in human serum at 37°C (Reynolds-Braude phenomenon) was done (Figure 5). The suspected strains of Candida isolates were grown on cornmeal agar (CMA) by incubating at 25°C for 2-3 days and observed for chlamydospore formation (Figure 6).

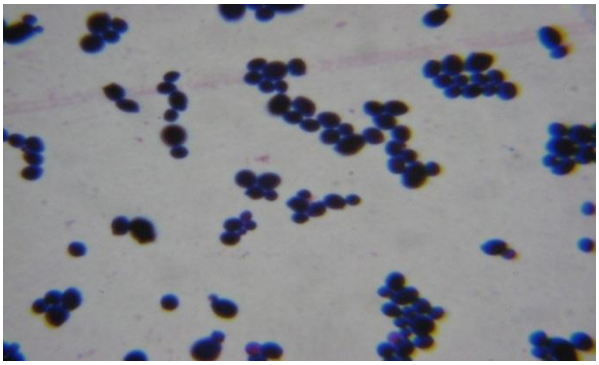

Figure 4: Gram stain smear from growth on SDA.

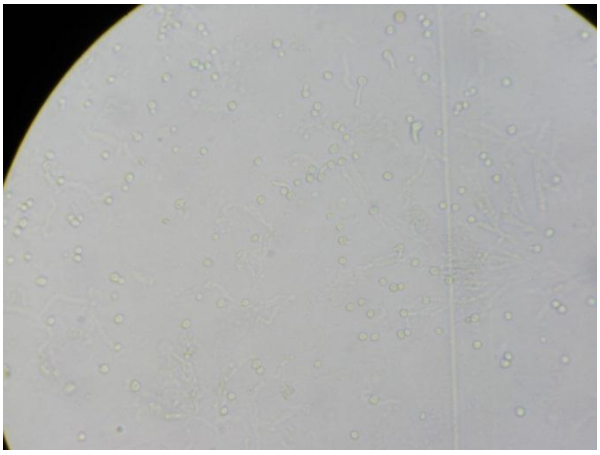

Figure 5: Germ tube test in Candida albicans.

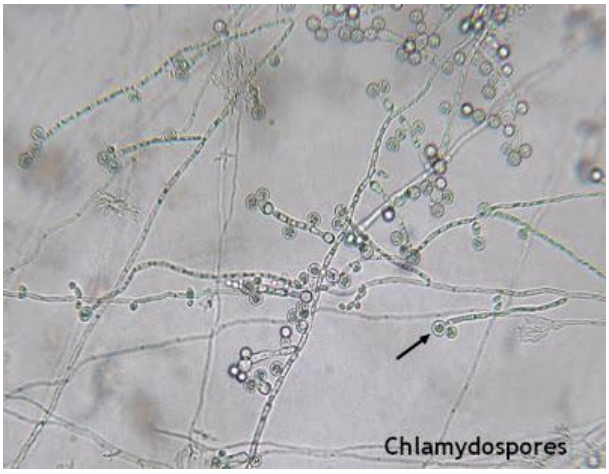

Figure 6: Chlamydospore of C. albicans on cornmeal agar.

For species identification, the isolates from the Sabouraud's dextrose agar medium were inoculated on CHROM agar medium and incubated at 37°C in dark for 48 hours.[5] Only pigmented colonies were considered for species identification.

- C. albicans - Light green
- C. dubliniensis - Dark green
- C. tropicalis - Blue
- C. glabrata - Pink to purple
- C. krusei - Pink
- C. parapsilosis - Cream to pale pink

Figure 7: CHROM agar showing coloured colonies of Candida species.

Antifungal susceptibility testing was done by disk diffusion method as per CLSI guidelines (M44-A). It recommends the use of Mueller-Hinton agar supplemented with 2% glucose and 0.5µg/ml methylene blue dye medium. Inoculum was prepared from the yeast grown on SDA for 24hrs, adjusted to match the turbidity of 0.5 Mc Farlands standard in the spectrophotometer. Sterile applicator swab was moistened in that cell suspension and used to inoculate the surface of Mueller-Hinton agar. Antifungal discs were placed and incubated in BOD for 24hrs and observed for zones of inhibition.

Antifungal discs used were:

- Amphotericin B - 20 µg
- Itraconazole - 10 µg
- Fluconazole - 10 µg
- Ketoconazole - 10 µg
- Clotrimazole - 10 µg
- Nystatin - 100 units/disc

- For azoles, sensitivity zones were:

- Susceptible - when ≥17 mm diameter
- Intermediate - in between 14 mm -16 mm diameter
- Resistant - when ≤13 mm diameter.

- For Amphotericin B, sensitivity zones were:

- Susceptible - when ≥15 mm diameter
- Intermediate – in between 13 mm -14 mm diameter
- Resistant - when ≤12 mm diameter.

RESULTS

Majority of patients were between 51-60 years (22%) of age followed by 41-50 years of age (9%) and 61-70 years (5%) (Table 1).

Table 1: Age wise distribution of cases.

Age (years)	Chemotherapy		Radiotherapy	
	No.	Culture positive	No.	Culture positive
21-30	2	Nil	-	-
31-40	6	1	8	1
41-50	10	3	16	6
51-60	22	10	20	12
61-70	10	2	6	3
Total	50	16	50	22

In study group of the 100 samples tested, 38% were culture positive for Candida. Majority of culture positives were from male patients, 24 out of 38 culture positives were from both chemotherapy and radiotherapy cases. Out of 38 culture positive samples, 22 (57.9%) were from RT patients and 16 (42.1%) were from the CT patients. Majority of patients were of carcinoma larynx and oral cavity (Table 2).

Table 2: Gender wise distribution.

Gender	Chemotherapy		Radiotherapy		Total
	Culture positive	Culture negative	Culture positive	Culture negative	
Males	10	18	14	16	58
Females	6	16	8	12	42
Total	16	34	22	28	100

The most commonly isolated species in both chemotherapy and radiotherapy patients is C. albicans 60.5% (23), followed by non albicans species, C. tropicalis 18.4% (7), C. glabrata 10.5% (4), C. krusei 7.9% (3), C. parapsilosis 2.6% (1) (Table 3).

Table 3: Candida speciation both in RT & CT patients.

Species	CT patients	RT patients	Total
C. albicans	10	13	23 (60.5%)
C. tropicalis	3	4	7 (18.4%)
C. glabrata	3	1	4 (10.5%)
C. krusei	0	3	3 (7.9%)
C. parapsilosis	0	1	1 (2.6%)
Total	16	22	38

In the control group, 9 (18%) out of 50 samples were culture positive and all the isolates were Candida albicans (Table 4).

Table 4: Control group (n=50).

Culture positive	Culture negative	Total
9 (18%)	41 (82%)	50

In the present study susceptible as well as intermediate sensitive were taken as sensitive (≥13 mm) and resistant when ≤12 mm. All the Candida species showed sensitivity to nystatin, amphotericin B, itraconazole and ketoconazle with zones ≥13 mm and relative resistance to Fluconazole and clotrimazole with zones ≤12 mm in the study group. In the control group all the Candida albicans were sensitive to fluconazole.

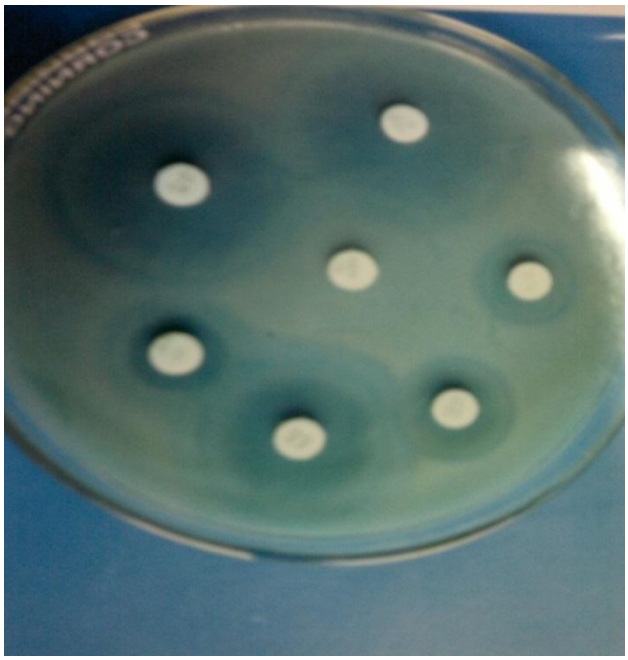

Figure 8: Antibiotic susceptibility by Disk diffusion method.

DISCUSSION

Opportunistic fungal infections are increasing as there is increase in the number of immunocompromised individuals due to CT and RT for malignancies, increased number of patients undergoing transplantations, prolonged hospital stay in intensive care units, metabolic disorders like diabetes, HIV.[6,7]

Radiation induced fragility of oral mucosa which leads to erythematous lesions, ulcerations and compromised salivary function is thought to be a major contributing factor for oral candidiasis. Suppression of antifungal activity of leukocytes and increase in candidal cell virulence are the predisposing factors for candidiasis in CT & RT undergoing patients.[8]

The increased number of non albicans species may be due to changing ecology of the pathogens and evolution of non albicans species as pathogens in immunocompromised patients.

In the present study Candida albicans was the most common isolate 23 (60.5%) out of 38 isolates[9,10] which correlates with the studies of Amador et al.[11] who reported 68%, Shaheen & Taha[12] reported 56% and Hema Suryawanshi et al.[13] reported 84.62% of C. albicans.

In the present study the non albicans species isolated were 15 (39.5%) out of 38 isolates which correlates with Shaheen & Taha[12] who reported 44% and Amador et al.[11] who reported 32%.

In the present study the non albicans species isolated were C. tropicalis, C. glabrata, C. krusei, C. parapsilosis which correlates with Redding et al.[14] and Dahia et al.[15] Among non albicans species C. tropicalis was the common isolate 7 (46.7%) out of 15 non albicans species which correlates with Shoba Rani Bakki et al.[16]

In the control group C.albicans was isolated in 9 (18%) out of 50 which correlates with Shoba Rani Bakki et al.[16] who reported 22% of controls and Cohen et al.[17] who reported 20-60% of controls.

In the present study the prevalence of candida is more in males (63.1%) when compared to females which correlate with Mohd Suhail Lone et al.[18] who reported 54% in males and Shoba Rani Bakki et al.[16] and Gooris et al.[19]

All candida species isolated were sensitive to nystatin, ampotericin B, itraconazole and ketoconazole in the present study. All the Candida isolates were resistant to clotrimazole and fluconazole.[20-22] In the control group all the C. albicans were sensitive to Fluconazole which correlates with studies of Mohd Suhail Lone et al.[18]

CONCLUSIONS

Increase in frequency of oral candidiasis in patients undergoing chemotherapy and radiotherapy was observed. This increase in positivity may be attributed to inadequate nutritional status and poor oral hygiene during chemotherapy and radiotherapy. Culture positivity is more in RT patients than in CT patients and more in males than in females. Though C. albicans is the predominant isolate, non albicans species are also emerging. All the Candida species isolated from study group were sensitive to nystatin, amphotericin B, itraconazole and ketoconazle. In the study group all the Candida species were resistant to clotrimazole and fluconazole and in the control group, all the C. albicans were sensitive to fluconazole.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the institutional ethics committee

REFERENCES

1. Samaranayake LP, MacFarlane TW, Lamey PJ, Ferguson MM. Comparison of oral rinse and imprint sampling technique for the detection of the yeast, coliform and staphylococcus aureus carriage in the oral cavity. J Oral Pathol. 1986;15:386-8.
2. Ueta E, Tanida T, Yoneda K, Yamamoto T, Osaki T. Increase of Candida cell virulence by anticancer drugs and irradiation. Oral Microbiol Immunol. 2001;16:243-9.
3. Jham BC, França EC, Oliveira RR, Santos VR, Kowalski LP, da SilvaFreire AR. Candida oral colonization and infection in Brazilian patients undergoing head and neck radiotherapy: a pilot study. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2007;103:355-8.
4. Milne LJ. Fungi ML, Mackie, Mc Cartney. Chapter 41. In: Collee JG, eds. Practical Medical Microbiology. 14th ed. Edinburgh: Churchill Livingstone; 1996: 695-717.
5. Jagadish Chander. Text book of mycology. In: Jagadish Chander, eds. A Book. 3rd ed. Pune, India: Mehta publishers; January 2009.
6. Belazi M, Velegraki A, Koussidou-Eremondi T, Andreadis D, Hini S, Arsenis G, et al. 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;19:347-51.
7. Jeyanthi Premkumar, Pratibha Ramani, Thind Infection Ruvengadam Chandrasekhar. Detection of species diversity in oral candida colonization and anti-fungal susceptibility among non-oral habit adult diabetic patients. J Natural Sci Biol Med. 2014;5(1):148-54.
8. Ueta E, Tanida T, Osaki T. A novel bovine lactoferrin peptide, FKCRRWQWRM, suppresses Candida cell growth and activates neutrophils. J Pept Res. 2001;57:240-9.
9. Redding SW, Bailey CW, Lopez-Ribot JL, Kirkpatrick WR, Fothergill AW, Rinaldi MG, et al. Candida dubliniensis in radiation induced oropharyngeal Candidiasis. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2001;91:659-62.
10. Nicolatou-Galitis O, Dardoufas K, Markoulatos P, Sotiropoulou-Lontou A, Kyprianou K, Kolitsi G, et al. Oral pseudomembranous candidiasis, herpessimplex virus-1 infection, and oral mucositis in head and neck cancer patients receiving radiotherapy and granulocyte-macrophage colony stimulating factor (GM-CSF) mouthwash. J Oral Pathol Med. 2001;30:471-80.

11. Ramirez-Amador V, Silverman S Jr, Mayer P, Tyler M, Quivey J. Candidal colonization and oral candidiasis in patients undergoing oral and pharyngeal radiation therapy. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 1997;84:149-53.
12. Shaheen MA, Taha M. Species identification of Candida isolates obtained from oral lesions of hospitalised and non-hospitalized patients with Oral Candidiasis. Egyptian Dermatol J. 2006;2:14-8.
13. Hema Suryawanshi, Sindhu M. Ganvir, Vinay K. Hazarey. Oropharyngeal candidosis relative frequency in radiotherapy patient for head and neck cancer. J Oral Maxillofac Pathol. 2012;16(1):31-7.
14. Redding SW, Dahiya MC, Kirkpatrick WR, Coco BJ, Paerson TF, Fothergill AW, et al Candida glabrata is an emerging cause of oropharyngeal Candidiasis in patients receiving radiation for head and neck cancer. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2004;97:47-52.
15. Dahiya MC, Redding SW, Dahiya RS, Eng TY, Kirkpatrick WR, Coco BJ, et al. Oropharyngeal candidiasis caused by non-albicans yeast in patients receiving external beam radiotherapy for head and neck cancers. Int J Radiat Oncol Biol Phys. 2003;57:79-83.
16. Shoba Rani Bakki, Lalith Prakash Chandra Kantheti. Candidal carriage, isolation and species variation in patients undergoing radiotherapy and chemotherapy for head and neck tumours. J Dr. NTR Univ Health Sci. 2014;3(1):28-34.
17. Cohen R, Roth FJ, Delgado E, Ahearn DG, Kalser MH. Fungal floral of the normal human small and large intestine. N Engl J Med. 1969;280:638-41.
18. Mohd Suhail Lone, Gulnaz Bashir, Nargis Bali, Syed Sajad, Shieh Aejaz, Humaira Bashir, et al. Oral candida colonisation and their infection in cancer patients and their antifungal susceptibility in a tertiary care hospital. Int J Adv Res. 2014;2(5):541-50.
19. Gooris PJ, Maat B, Vermey A, Roukema JA, Roodenburg JL. Radiotherapy for cancer of the lip: a long-term evaluation of 85 treated cases. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 1998;86:325-30.
20. Kumar CPG, Sundararajan T, Menon T, Venkatadesikalu M. Candidosis in children with onco-hematological disease in Chennai, South India. Jpn J Infect Dis. 2005;58:218-21.
21. Colombo AL, Da Matta D, De Almeida LP, Rosas R. Fluconazole susceptibility of Brazilian Candida isolates assessed by a Disk diffusion method. Braz J Infect Dis. 2002;6:118-23.
22. Meis J, Petrou M, Bille J, Ellis D, Gibbs D; Global Antifungal Surveillance Group. A global evaluation of the susceptibility of Candida species to fluconazole by disk diffusion. Diagn Microbiol Infect Dis. 2000;36:215-23.

DOI: 10.5455/2320-6012.ijrms20150529
Cite this article as: Yogitha PVV, Lakshmi N, Lakshmi KR, Krishna PBM, Cheemala SS. Isolation and speciation of genus candida in patients undergoing chemotherapy and radiotherapy for head and neck tumours. Int J Res Med Sci 2015;3:1189-94.