Non *Candida albicans Candida* (NCAC) of oral cavity in head and neck cancer patients under cancer therapy: Prevalance, species identification and antimycotic sensitivity pattern

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Abstract

Introduction: Cancer patients remain at risk for developing serious infections due to oropharyngeal *Candida* colonisation as the incidence of Candidiasis continues to rise after chemotherapy (CT), Radiochemotherapy (RCT) or Radiotherapy (RT) for head and neck cancer. So, accurate and rapid identification of *candida* species is very important in clinical laboratory.

Objective: To study the prevalance, species identification and antimycotic sensitivity pattern of *Candida spp*. of oral cavity in patients of head & neck cancer undergoing chemotherapy (CT) & Radiochemotherapy (RCT) and compare with control group.

Materials and Methods: This is prospective cross sectional and case control analysis including 110 patient in study group; chemotherapy (CT)-55 patient, Radiochemotherapy (RCT)-55 patients and 50 healthy individuals as control. *Candida* isolates identified by standrad methods using morphological, biochemical & chromogenic property of different *Candida spp.* and antimycotic sensitivity applied by disc diffusion method.

Results: Prevalence of *Candida spp*. colonization seen in 50% patients of study and 20% in control group (p value- 0.004) ; patients on RCT (63.6%) have higher prevelance as compare to CT (36.4%), (p 0.007). *C. tropicalis* was most common (38%) species followed by C. *albicans* (11%) & *C. glabrata* (11%); most of isolates were sensitive to Amphotericin B and Nystatin and least sensitive to Fluconazole.

Conclusion: High prevalence of Candidal carriage and the species variation and changing pattern of antifungal susceptibility of *Candida spp* in patients undergoing chemotherapy and radiation therapy require isolation and speciation of the causative *Candida* sp. and prophylaxis is needed to prevent infection among these patients.

Keywords: *Candida species, Non Candida albicans Candida* (NCAC), Oral cavity, Head & neck cancer, Radiochemotherapy.

Introduction

Opportunistic fungal infections, mainly Candidiasis, is common in immunocompromised patients such as those undergoing chemotherapy or Radiotherapy or both Radiochemotherapy (RCT). Opportunistic fungal infections occur in a host whose immunological defense mechanism is weakened by endogenous causes like cancer, diabetes, or exogenous causes like immune-suppressive drug therapy by nonpathogenic fungi.[1] Patients receiving therapy for head and neck cancer are particularly susceptible to oropharyngeal candidiasis.[2-3] *Candida* species are usually normal oral commensals and but they can be a opportunistic pathogen, depands on virulence of the organism and also the host factors.[4] There is many Candidal species like *C. albicans*; *C. tropicalis*; *C. krusei*; *C. glabrata*; *C. guillermondii*; *C. parapsilosis*; *C. kefyr*/*pseudotropicalis*; *C. dubliniensis*; *C. viswnathii*; *C. stellatoidea,* and so on. Various past studies have shown that *Candida albicans* has been most predominant species isolated in patients receiving cancer therapies but recently there is an increase in non *Candida albicans Candida* (NCAC). It seems that these NCAC are responsible for increase in resistance to antifungal drugs. Apart from this, the antifungal susceptibility patterns of *C.albicans* and also the newer species of *Candida* has been changing. Hence, the isolation and speciation of the causative species of *Candida* is gaining importance.[5-6]

With this as the back ground, a study was done attempting to find out the Candidal carrier state and the species variation in patients undergoing chemotherapy and radiation therapy for head and neck cancers.

Aims and Objectives

To study the prevalence of *Candida*, speciation and antifungal susceptibility testing for different *Candida* species by Disk diffusion method in patients undergoing chemotherapy and Radiochemotherapy for head and neck tumors.

Materials and Methods

Ethics: Ethical clearance for the study was obtained from Local Ethical clearance Committee of Dr. S. N. Medical College, Jodhpur, Rajasthan.

Study Design: The present study was carried out in the Dept. of Microbiology, S.N. Medical College, Jodhpur, Rajasthan, India from January 2015 to July 2015. Study group comprised of total 110 patients of head and neck tumours of age 20-80 years - 55 patients on chemotherapy (CT) and 55 on radiochemotherapy (RCT). 50 normal healthy individuals were taken as control group, patient attendants whose age and sex were matched with study groups and were apparently

healthy with no systemic diseases.

Exclusion Criteria: The patients with other risk factors for Candidiasis such as diabetes, recent usage of corticosteroids or antibiotics as well as patients using intra oral prostheses and patients who had received antifungal therapy were excluded from the study.

Sample Collection & Processing: The samples were collected from control group and 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. Sabouraud's Dextrose Agar (SDA) with gentamycin was used as primary culture media 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. The colonies were further subjected to HiCrome candida differential Agar,[7] germ tube test, carbohydrate fermentation test, and corn meal tween 80 agar streak culture to identify various species of *Candida*.[8] 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. The colour of various Candida sp. on Hi Crome agar is following

1. *C. albicans* - Light green
2. *C. dubliniensis* - Dark green
3. *C. tropicalis* - Blue
4. *C. glabrata* - Pink to purple
5. *C. krusei* – Rose Pink
6. *C. parapsilosis* - Cream to pale pink

Antifungal susceptibility testing was done by disk diffusion method as per CLSI guidelines (M44-A).[9] 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 and 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, Voricazole-1 µg, Nystatin - 100 units/disc.

Results

Total no. of patient were 110 in study group in them predominant population were male 80 (72.8%). *Candida* sp. were mostly isolated from age group 61-70 year (29%) followed by 51-60 year (27.2%). But results were non-significant with age (p value-0.38). (Table 1) The cancer of head and neck region were squamous cell carcinoma of Larynx (25.5%), Pharynx (20%), Tongue (14.6%), buccal mucosa (12.8%), Tonsil (11%), Upper Oesophagas (10%) & Palate (6%).

Candida spp. was more commonly isolated from study group (50%) i.e. patient on RCT (63.6%) and on CT (36.6%) as compared to control group (20%) (Table 2) and *C. tropicalis* was more prevalent spp in study group when compared to control group. (p 0.01). (Table 2)

NCAC was more common in study group- CT, RCT (90%, 88.5%) than *C. albicans* (10%, 11.5%) (p value <0.0001); While in control group both found equally. (Table 3)

All *Candida* isolates showed 80-100% sensitivity to Amphotericin B & Nystatin.

In the present study, *C. albicans* isolates were 83.3 % susceptible to Amphotericin B and Nystatin and showed 50 to 66% resistance to azoles. The *Candida tropicalis* isolates were 85.8% susceptible to Amphotericin B and showed 33 to 38 % resistance to azoles. The *Candida krusei* isolates were 100 % susceptible to Amphotericin B and showed 75 to 100 % resistance to azoles and *C. glabrata* isolates were 83.4 % susceptible to Amphotericin B and showed 50 to 66.7% resistance to azole. (Table 4)

In study group *C. albicans* showed higher resistance for azoles than polyene antifungal as compared to NCAC but results were non- significant. (Table 5) In control group there was not much difference in resistance profile between *C. albicans* and NCAC for most of antifungal agents and the results were non- significant. (Table 6)

Table 1: Age wise distribution of organism isolates in study group

Age	CT group Total *Candida* (20)	RCT group Total *Candida* (35)	Total (55)
<-30	1	1	2(3.6%)
31-40	2	1	3(5.4%)
41-50	5	7	12(21.8%)
51-60	5	10	15(27.2%)
61-70	5	11	16(29%)
> 70	2	5	7(12.7%)

[CT vs RCT p=0.38]

Table 2: Frequency of *Candida* spp. in study and control group

***Candida* spp.**	**Study Group**				**Control (50)**	**p value Study vs. Control**
	CT (55)	**RCT (55)**	**Total (110)**	**p value CT vs. RCT**		
C. albicans	2(10%)	4(11.4%)	6(11%)	.678	5(50%)	.321
C. dubliniensis	1(5%)	4(11.4%)	5(9%)	.363	0	.325
C. glabrata	2(10%)	4(11.4%)	6(11%)	.678	1(10%)	.321
C. guilliermondii	1(5%)	2(5.7%)	3(5.5%)	1.00	1(10%)	1.00
C. krusii	2(10%)	2(5.7%)	4(7.2%)	1.38	0	.310
C. parapsillosis	3(12%)	2(5.7%)	5(9%)	1.00	1(10%)	.666
C. pseudotrop.	3(15%)	2(5.7%)	5(9%)	1.00	0	.325
C. tropicalis	6(30%)	15(43%)	21(38%)	.050	2(20%)	.010
Total	20(36.6%)	35(63.6%)	55(50%)		10(20%)	

Table 3: Frequency *Candida albicans* and *Non Candida albicans Candida* (NCAC) in Study Group and Control

Organism	**Study group**		**Total**	**Control**
	CT group	**RCT group**		
C. albicans	2(10%)	4(11.5%)	6(11%)	5(50%)
NCAC	18(90%)	31(88.5%)	49(89%)	5(50%)
Total *Candida* spp.	20	35	55	10

[*Candida albicans* vs *Non Candida albicans Candida* (NCAC) P <0.0001]

Table 4: Antimycotic Resistance pattern of Candida species of study group

Drug / Organism	**AP**	**NS**	**FLC**	**CC**	**KT**	**VC**	**IT**
C. albicans (6)	1 (16.6%)	1 (16.6%)	4 (66.7%)	4 (66.7%)	4 (66.7%)	4 (66.7%)	3 (50)
C. dublineinsis (5)	2 (40)	2 (20)	2 (40)	3 (60)	2 (40)	2 (40)	2 (40)
***C. glabrata* (6)**	1 (16.6)	1 (16.6)	4 (66.7)	2 (33.3)	3 (50)	3 (50)	3 (50)
C. *guilliermondii* (3)	1 (33.3)	1 (33.3)	1 (33.3)	1 (33.3)	1 (33.3)	1 (33)	1 (33)
***C. krusii* (4)**	0 (0)	1 (25)	4 (100)	4 (100)	3 (75)	3 (75)	3 (75)
***C. parapsillosis* (5)**	0 (0)	0 (0)	3 (60)	3 (60)	2 (40)	2 (40)	2 (40)
***C.pseudo-tropicalis* (5)**	0 (0)	0 (0)	2 (40)	2 (40)	2 (40)	2 (40)	2 (40)
***C. tropicalis (*21)**	3 (14.2)	2 (9.5)	8 (38)	7 (33.3)	7 (33.3)	8 (38)	8 (38)

Table 5: Comparative analysis of Antimycotic Sensitivity pattern of Candida albicans vs NCAC of study group

Organism / Drugs		***C. albicans(6)***	**NCAC(49)**	**p value**
Amphotericin B	S	5(83.3)	42(85.7)	0.875
	R	1(16.6)	7(14.2)	
Nystatin	S	5(83.3)	42(85.7)	0.875
	R	1(16.6)	7(14.2)	
Itraconazole	S	3(50)	28(57.2)	0.739
	R	3(50)	21(42.8)	
Fluconazole	S	2(33.3)	25(51.1)	0.699
	R	4(66.7)	24(48.9)	
Ketoconazole	S	2(33.3)	29(59.2)	0.44

	R	4(66.7)	20(40.8)	
Clotrimazole	S	2(33.3)	27(55.1)	0.565
	R	4(66.7)	22(44.9)	
Voricazole	S	2(33.3)	28(57.2)	0.502
	R	4(66.7)	21(42.8)	

Table 6: Comparative analysis of Antimycotic Resistance pattern of Candida albicans vs NCAC of Control Group

Organism Drugs		*C. albicans (5)*	NCAC (5)	p value
Amphotericin B	S	5(100)	5(100)	0.00
	R	0(0)	0(0)	
Nystatin	S	5(100)	5(100)	0.00
	R	0(0)	0(0)	
Itraconazole	S	4(80)	4(80)	0.00
	R	1(20)	1(20)	
Fluconazole	S	4(80)	3(60)	0.490
	R	1(20)	2(40)	
Ketoconazole	S	4(80)	4(80)	0.00
	R	1(20)	1(20)	
Clotrimazole	S	4(80)	4(80)	0.00
	R	1(20)	1(20)	
Voricazole	S	3(60)	3(60)	0.00
	R	2(40)	2(40)	

Discussion

Due to chemotherapy and radiation therapy, patients have a greater predisposition towards mucositis and oral candidiasis. In the immune-compromised individuals, the members of the normal flora may probably acquire invasiveness and can become pathogenic. Earlier, *C. albicans* was the predominant isolate but now a days Non albicans species also have been proven to be emerging opportunistic pathogen. All may cause a similar spectrum of disease but differences in disease severity, epidemiology, virulence, and susceptibility to antifungal agents are seen. Due to this changing pattern, such studies involving isolation and identification of various species of *Candida* in immune compromised individuals is important for better treatment strategies.

In the present study, majority patients were male (72.8%) and in the age group of 51-60 years (32.7%) which is accordance to study done Bakkhi SR et al[1] Pangal M et al[10] Kamath MP et al.[11]

In the present study, colonization of *Candida* spp. in oral cavity was seen in study group (50%) among them 63.6% were from the RCT group. Yogitha PPV et al.[12] showed, in study group 38% and in control group 18% were culture positive for *Candida* spp. Bakkhi SR et al[1] also found there is high prevelance of *Candida* coloniation in study group (38%) in compare to control group (22%). This is in accordance to my study. The proportion of *C. albicans* in study group i.e. chemotherapy(CT) and Radiochemotherapy (RCT) cases showed a wide variation ranging from 17 to 52.5% in studies of Redding et al,[2] Ramirez et al,[4] Jham et al,[13] Nicolatou-Galitis et al,[14] l, Bag J et al,[15] Davies AN et al.[16] The study of Suryawanshi H[17] showed that *C. albicans* was seen in 78.57%, which was contrast to our study that showed NCAC was the predominant organism i.e. 49(89%).

Our study showed that NCAC were isolated at a higher rate (89%) than *C. albicans* (11%), which was in agreement with the findings of the studies by Mokaddas et al[18] (60.5%,39.5%), Chakrabati A[19] (75%,25%), Dharwad S[20] (53%,47%), Patel LR[21] (62.6%,37.4), Kashid RA[22] (70.7%,29.3%), Manchanda V[23] (72.4%, 27.6%), Jain M[24] (90%,10%) who also showed that NCAC incidence to be higher than that of *C. albicans.* These findings seem to suggest that non-albicans Candida are emerging as important pathogens and shift from *C. albicans* towards NCAC species during the cancer therapy, may be due to changing ecology of the pathogens, resistance profile and evolution of new species as pathogens in immunocompromised patients.

Among non albicans species, *C. tropicalis* was the most common isolate i.e. 42.8% which is accordance to studies Yogitha PPV(18.4%), Chakrabarti's A (42%), Jain M (42.85%).

The susceptibility pattern of all *Candida* isolates showed that overall azole resistance was 42-49% in NCAC & 50-67% in *C. albicans*. However, Amphotericin B and Nystatin was the most sensitive used polyene antifungal both in NCAC & *C. albicans.*

High susceptibility to Amphotericin B and Nystatin and increasing resistance to azole antifungal is also shown by various species of *Candida* also reported by studies of Dharwad S,[20] Xu Y[25] Lee JS.[26]

Dharwad S showed *C. albicans* isolates were 100% susceptible to Amphotericin B 22% resistance to

Fluconazole. The *Candida tropicalis* isolates were 87.5% susceptible to Amphotericin B & 25% resistance to Fluconazole. The *Candida krusei* isolates were 80% susceptible to Amphotericin B, showed 60% resistance to Fluconazole. The *C. glabrata* isolates were 100% susceptible to Amphotericin B and showed 66.66% resistance to Itraconazole and 33.33% resistance to Fluconazole.

Yonghao Xu et al's study showed *C. krusei* showed 40% resistance to Fluconazole. Study by Jin-Sol Lee et al. showed that *C. glabrata* was 38% resistance to Itraconazole and C. krusei showed 100% resistance to Fluconazole. *C. krusei* showed high resistance to Fluconazole due to their innate resistance to the drugs.

The extended prophylactic use of fluconazole in suspected cases would be a probable cause of high resistance pattern to fluconazole and major cause of NCAC dominance in our institute. The finding correlate with study done by Roy R et al,[27] Kothavade et al.[28] Adhikary et al.[29] So, Antifungal drugs should be used as high doses only for the treatment of oral candidiasis, not for prophylaxis.

Conclusion

Patients with head & neck malignancy are often immune supressed and cancer therapy induced oral mucositis and a consequently reduced ability to maintain oral hygiene also increase the risk for oral candidiasis in both chemotherapy & radiation therapy populations. Previously, *Candida albicans* was most common species involved but now a days, other species, such as *Candida tropicalis* and *Candida glabrata*, also present in a clinically significant proportion of patients. This is important because non-albicans *Candida* species (NCAC), especially *Candida tropicalis*, are more likely to spread into the systemic circulation. Recent studies have shown other species of *Candida* also to be emerging pathogens. Apart from this, the antifungal susceptibility patterns of *C. albicans* and also the newer species of *Candida* has been changing. Hence, the isolation and speciation and antimycotic sensitivity of the causative species of *Candida* is gaining importance.

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