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ORIGINAL ARTICLE

Changes in Candida Profile in Patients Undergoing Intensity Modulated Radiotherapy for Head and Neck Malignancies

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ABSTRACT

The increased oral *Candida* colonization that resulted for radiotherapy often leads to candidiasis. Intensity Modulated Radiotherapy (IMRT) is a technique of delivering radiation with improved dose distributions sparing the surrounding normal tissue and decreasing the ill-effects. **Objective:** To identify and quantify changes in the *Candidal* carriage of patients undergoing IMRT for head and neck malignancy. **Methods:** Saliva from 37 patients undergoing IMRT for head and neck malignancy was collected. The *Candida* species profile pre- and post-IMRT was evaluated using semi quantitative fungal culture. The changes in the distribution of the growth of *Candida* species due to IMRT was analyzed using Wilcoxon sign rank test. **Results:** Twenty-two patients were *Candida*-positive pre-IMRT, while 24 patients were *Candida*-positive post-IMRT. *Candida* species isolates pre-IMRT were *C. albicans* (63%), *C. tropicalis* (26%), *C. glabrata* (7%), *C. krusei* (4%) and post-IMRT, were *C. albicans* (55%), *C. tropicalis* (30%), *C. glabrata* (12%) and *C. krusei* (3%). *C. albicans* showed increased growth post-IMRT in the range of 10³ to 10⁴ colony-forming units per ml of saliva (p>0.05). **Conclusion:** There was no significant effect of IMRT on the distribution of growth of *Candida*. *Candida albicans* was the most common species. A change towards non *C. albicans* species post-IMRT was seen.

Keywords: *Candida*, head and neck malignancy, IMRT, radiotherapy

INTRODUCTION

Candida species are a normal constituent of the human oral micro-flora. They are naturally harmless, but become pathogenic when the host immune barriers are violated. Recently, their increase in pathogenic state has been observed, this could be attributed to various medical conditions and their interventions. One such reason is oral cancer and its treatment protocols.¹

Cancer is a multifactorial disease in which a cell or a group of cells display uncontrolled growth, invasion, and sometimes metastasis. The term head and neck malignancy refers to a group of cancers with similarities in their biological background, originating from the upper aero digestive tract, including the lip, oral cavity, nasal cavity, paranasal sinuses, pharynx, and larynx.^{2,3}

Radiation therapy is the most common form of treatment administered to patients with such malignancies. There are different forms of radiation therapy, including 3D Conformal Radiation Therapy (3D CRT), Intensity-Modulated Radiation Therapy (IMRT), and brachytherapy, which are the commonly employed modalities of treatment for malignancies of the head and neck.⁴

The effects thus seen in the oral cavity due to radiotherapy include mucositis, xerostomia, radiation caries, dysgeusia, candidial infections- all leading to an altered dietary intake, thus, resulting in malnutrition which further decreases the general health in an already diseased human being.^{5,6} The purpose of this study was to identify and quantify *Candida* species in saliva collected from patients undergoing IMRT for head and neck malignancy and to see the change that IMRT brought about to the *Candidal* carriage in such patients.

METHODS

In the present study, thirty-seven patients were included. All these patients were receiving IMRT for a head and/ or neck malignancy. None of the patients were taking antibiotics and antifungals during the study and patients who had a systemic illness that would make them more prone for *candidial* infections were excluded from the study. Ethical clearance for the study was obtained from the Institutional Ethics Committee. After informing the patients regarding the nature of the study and after obtaining written consent, saliva was collected from all the patients for *Candida* culture procedures at baseline, i.e. the day of the commencement of radiotherapy and on the last day of their treatment protocol immediately after radiotherapy, by asking them to spit into a sterile container provided. The patients received a total dose of 50.4-70 gray. This dose was divided into fractions ranging from 28-39 fractions.

Semi-quantitative culture was done for the saliva specimen.⁷ About 10µl of saliva was streaked onto the culture plate using a four-millimetre nichrome loop on commercial media, Sabouraud’s dextrose agar (HiMedia Laboratories, Mumbai, India) and then on *Candida* CHROM agar (HiMedia Laboratories, Mumbai, India), for culture of fungi. The culture media was incubated at 37°C for 24-48 hours. After the incubation time, the fungi grown on the media was identified by Gram’s staining, Germ tube test and colony colour on CHROM agar as per manufacturer’s chart. Light green colonies were identified as *C. albicans*, blue colonies were identified as *C. tropicalis*, cream to white colonies were identified as *C. glabrata* and purple colonies were identified as *C. krusei*. The different colonies grown in 10µl of saliva were counted and converted further for 1000µl (One ml.) of saliva. The semi-quantitative colony count was categorized as <10³, 10³ to 10⁴, 10⁴ to 10⁵, 10⁵ to 10⁶ and >10⁶ colony forming units per ml (cfu/ml) of saliva.

RESULTS

The study consisted of 37 subjects who were undergoing IMRT for head and/ or neck malignancy. The mean age of the 37 patients was 50.54 years, 62.2% were male and 37.8% females (Figure 1). Most of the malignancies were those of the buccal mucosa, which affected 18.9% of the subjects; followed by carcinoma of the oropharynx, which was diagnosed in 16.2% of the subjects (Figure 2).

Out of the 37 patients, 22 (54.05%) patients had isolates of *Candida* pre-IMRT and 24 (67.56%) patients had isolation of *Candida* post-IMRT. Out of 22 patients who had positive *Candida* isolates pre-IMRT, 63% had *C. albicans*, 26% had *C. tropicalis*, 7% had *C. glabrata*, and 4% had *C. krusei* (Figure 3).

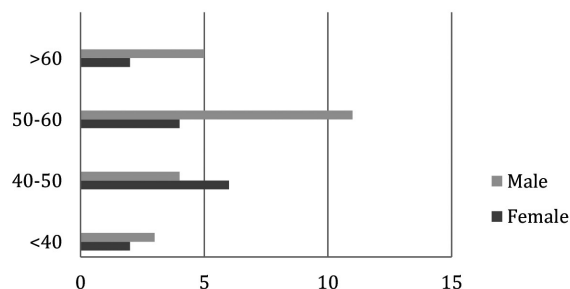


Figure 1. Distribution of subjects according to age and gender

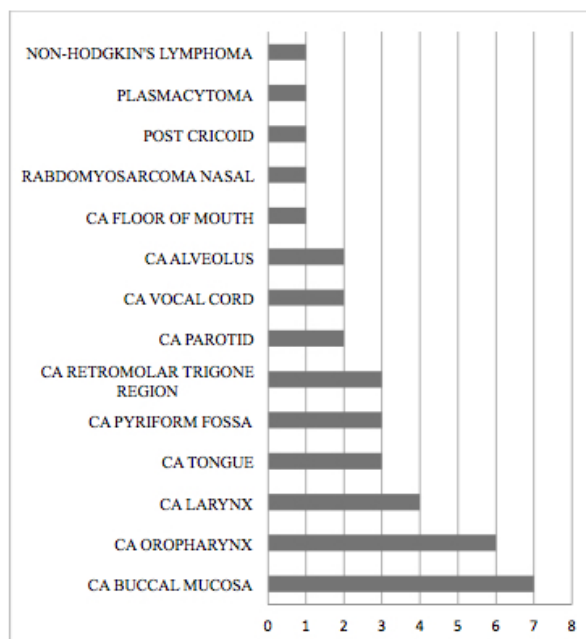


Figure 2. Type malignancies of patients receiving IMRT

Post-IMRT, out of 24 patients that had a positive isolation of *Candida*, 55% had isolates of *C. albicans*, 30% had isolates of *C. tropicalis*, 12% had isolates of *C. glabrata* and 3% had isolates of *C. krusei* (Figure 4 and 5).

Out of the 20 patients who showed no growth of *C. albicans* pre-IMRT, seven patients showed increased *C. albicans* growth post-IMRT out of which, 5 (25%) patients showed growth in the range of 10³-10⁴ cfu/ml and 2 (10%) patients showed growth in the range of 10⁵-10⁶ cfu/ml.

Similarly, out of the 30 patients who showed no growth of *C. tropicalis* pre-IMRT, nine patients showed increased growth post-IMRT out of which, 1 (3.3%) showed <10³ cfu/ml and 8 (26.7%) showed increased growth in the range of 10³-10⁴ cfu/ml and out of 35 patients that showed no growth of *C. glabrata* pre-IMRT, 5 (14.3%) patients showed increased growth in the range of 10³-10⁵ cfu/ml and out of 36 patients

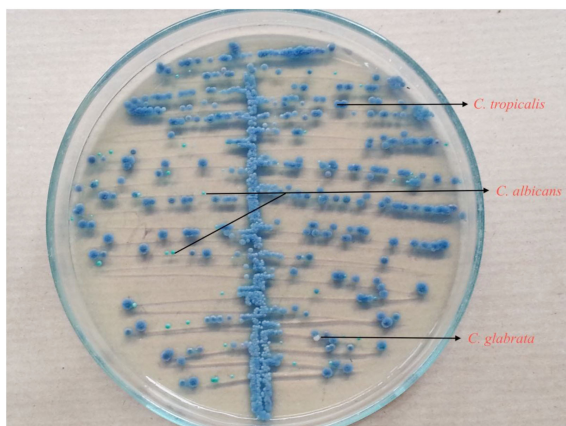


Figure 3. *Candida* species identification on *Candida* CHROM agar (HiMedia Laboratories, Mumbai, India)

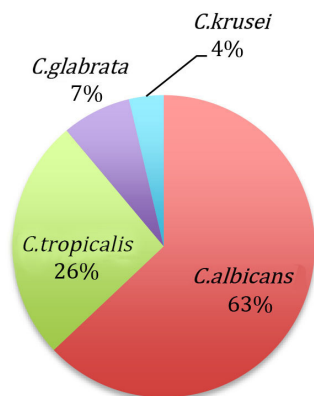


Figure 4. *Candida* species isolated from saliva before IMRT

that showed no growth of *C. krusei* pre-IMRT, only 1 (2.8%) showed increased growth in the range of 10^3 - 10^4 cfu/ml (Table 1).

Wilcoxon Sign rank test was done to assess the distribution of the growth of *Candida species* due to IMRT (Table 2). Only *C. albicans* showed an increased pattern of growth due to IMRT. Majority of the patients who showed no growth of *C. albicans* pre-IMRT, showed increased growth post-IMRT and this growth was in the range of 10^3 to 10^4 . But this change in the distribution of growth of *C. albicans* was not statistically significant ($p > 0.05$).

DISCUSSION

The Latin root word “candid” perfectly describes *Candida*, which means white. They are yeasts that are described under the fungus kingdom. *Candida* is a dimorphic fungus that can be found in yeast and hyphae forms, a property, which makes it retain its harmless commensal state or become invasive and turn pathogenic.⁸ Seen as a normal commensal in most of the mucosal surfaces, it becomes invasive and pathogenic

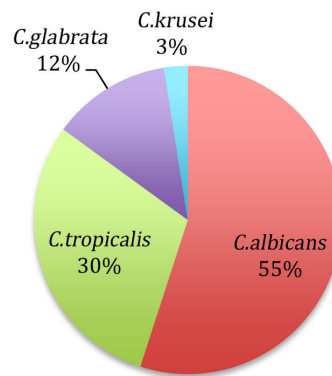


Figure 5. *Candida* species isolated from saliva after IMRT

only when there is a disruption in the balance of the internal environment.^{9,10}

Patients who have been irradiated to the head and neck region end up having xerostomia that ranges from mild decrease of saliva and inability to eat solid dry food to complete absence of saliva. The host capacity is decreased resulting in reduced clearance of *Candida* organisms, thus causing it to flourish. Antimicrobial properties of saliva, that normally keep the oral micro flora in check are also absent or diminished. Altered pH and buffering capacity of saliva also plays a role in candidiasis.^{11,12}

IMRT is a new and advanced technique of delivering radiation in a precise manner. This high-precision radiation therapy uses computer-controlled linear accelerator (linac) that delivers the dose to the tumor or specific areas that lie within the tumor. IMRT, makes it possible to generate dramatically improved dose distributions, that are tailored to fit complex shapes of the tumour and delivers optimum dose of radiation to the malignancy, sparing the surrounding normal tissue thus decreasing the ill effects of radiation that was seen with conventional radiotherapy.¹³⁻¹⁵

The amount of radiation delivered for a malignancy of the head and neck region usually ranges from 50-70 gray. Similar range of dose was also delivered to the patients in this study. Any dose above 52 gray can result in dysfunction of the salivary gland, resulting in xerostomia, which is seldom reversible and causes decreased clearance of oral *Candida* organisms, increasing its colonization making it pathogenic.^{16,17}

There are various species of *Candida* that can be isolated from the oral cavity; the most prominent ones are *C. albicans*, followed by *C. tropicalis* and *C. glabrata*. Previous study identified that *C. albicans* was isolated from more than half (59%) of the patients undergoing radiotherapy.^{18,19} This was also seen in our study where post-IMRT 55% of the *Candida* isolates were *C. albicans*. But the distribution of *C. albicans* post-IMRT was not statistically significant.

Table 1. The colony forming units of different candida species pre-IMRT and post-IMRT in one ml of saliva (CFU/ml)

Candida Species	Post-IMRT	Pre-IMRT				Total
		No growth N(%)	<10 ³ N(%)	10 ³ -10 ⁴ N(%)	10 ⁴ -10 ⁵ N(%)	
<i>C. albicans</i>	No growth	13(65)	1(50)	1(8.3)	0	15(40.5)
	<10 ³	0	0	2(16.7)	1(33.3)	3(8.1)
	10 ³ -10 ⁴	5(25)	1(50)	7(58.3)	1(33.3)	14(37.8)
	10 ⁴ -10 ⁵	0	0	2(16.7)	1(33.3)	3(8.1)
	10 ⁵ -10 ⁶	2(10)	0	0	0	2(5.4)
<i>C. tropicalis</i>	No growth	21(70)	1(100)	3(60.0)	0	25(67.6)
	<10 ³	1(3.3)	0	2(40.0)	0	3(8.1)
	10 ³ -10 ⁴	8(26.7)	0	0	1(100.0)	9(24.3)
<i>C. glabrata</i>	No growth	30(85.7)	0	2(100.0)	0	32(86.5)
	10 ³ -10 ⁴	3(8.6)	0	0	0	3(8.1)
	10 ⁴ -10 ⁵	2(5.7)	0	0	0	2(5.4)
<i>C. krusei</i>	No growth	35(97.2)	0	1(100.0)	0	36(97.3)
	10 ³ -10 ⁴	1(2.8)	0	0	0	1(2.7)

Table 2. Distribution of the growth of Candida species pre- and post-IMRT

Candida species	IMRT	N	Range	Median (Q1-Q3)	Wilcoxon Sign rank test	
					z	p-value
<i>C. albicans</i>	Pre	37	No growth - 10 ⁴ to 10 ⁵	No growth (No growth – 10 ⁴ to 10 ⁵)	-1.58	0.11(NS)
	Post	37	No growth - 10 ⁵ to 10 ⁶	10 ³ to10 ⁴ (No growth – 10 ³ to 10 ⁴)		
<i>C. tropicalis</i>	Pre	37	No growth - 10 ⁴ to10 ⁵	No growth (No growth – No growth)	-1.24	0.22(NS)
	Post	37	No growth – 10 ³ to 10 ⁴	No growth (No growth – 10 ³ to 10 ⁴)		
<i>C. glabrata</i>	Pre	37	No growth – 10 ³ to 10 ⁴	No growth (No growth – No growth)	-1.41	0.16(NS)
	Post	37	No growth - 10 ⁴ to10 ⁵	No growth (No growth – No growth)		
<i>C. krusei</i>	Pre	37	No growth – 10 ³ to 10 ⁴	No growth (No growth – No growth)	0.00	1.00(NS)
	Post	37	No growth – 10 ³ to 10 ⁴	No growth (No growth – No growth)		

p>0.05 Non significant, NS

Other study also put forth similar findings, where after *C. albicans*, the species isolated were *C.glabrata*, *C.krusei*, and *C.tropicalis*. This was also shown by a study by Dambroso D *et al.*, who established that radiotherapy is an important influencing factor for oral candidiasis.^{20,21} Our study also showed that Candida was seen to increase post IMRT.

It was showed that oral cavity colonization by non-Candida albicans Candida (NCAC) was seen in 71.4%

of the patients post radiotherapy. In our study, post-IMRT, 45% of the *Candida* species isolated was NCAC (*C. tropicalis*, *C. glabrata* and *C. krusei*). Although our study showed similar species isolation, the percentage isolated was much less, this could be attributed to the fact that some of the patients in the study by Jham BC *et al.*, were undergoing conventional radiotherapy as compared to our study where the patients were undergoing IMRT.²²

In a study on 37 patients receiving radiotherapy showed that, NCAC was emerging, as the new cause for Candidiasis and was seen in 30% of the cases, as was in line with our study and further went to say that Chromogenic media was most helpful in identifying these infections quickly and accurately.²³ Similarly in our study, *Candida species* was identified using Candida CHROM agar. This is a chromogenic media, which can identify more than one organism on a single medium by observing the distinct colour change in the medium, which allows for an easier and accurate differentiation.²⁰

Irradiation-induced xerostomia favors intraoral colonization of *Candida species*, especially *C. albicans*, as supported by a previous study which also stated that *Candida species* had an ability to adapt and increase its survival in the oral cavity, particularly when salivary defenses are weakened chiefly due to radiotherapy.²⁴ This was also in agreement with our study where the oral cavity was colonized mainly by *C. albicans*, although, the change in the pattern of growth of *C. albicans* was not statistically significant. Moreover, xerostomia was also seen in all of our patients, and thus collection of saliva became very difficult post-IMRT.

It identified that *C. glabrata* strains caused an infection in patients receiving radiotherapy.²⁵ In our study, out of the 35 patients that showed no isolates of *C. glabrata* pre-IMRT, 14.3% of the patients showed growth post-IMRT in the range of 10^3 to 10^5 cfu/ml of saliva, although this was not statistically significant, never the less, infections could arise from *C. glabrata*.

Increase in *Candida* isolates post-radiotherapy seen in a study by *Yogitha PVV et al.*, was attributed to poor oral hygiene and inadequate nutrition.²⁶ Although inadequate nutrition could be a reason due to mucositis and thus inability to eat, the subjects in our study were given oral prophylaxis prior to RT and the teeth with poor prognosis were extracted, hence, the reason of poor oral hygiene could be safely excluded from our study as a causative factor.

CONCLUSION

The most common species that was isolated in patients undergoing Intensity Modulated Radiotherapy for a head and neck malignancy was *C. albicans*, followed by *C. tropicalis*, *C. glabrata* and *C. krusei*. There was also a change seen towards Non *Candida albicans* *Candida species* post Intensity Modulated Radiotherapy.

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