

# An Assessment of Candidal Colonization and Species Differentiation in Head and Neck Cancer Patients Receiving Radiation

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## ABSTRACT

**Background:** Oral colonization with *Candida* species has been observed in upto 93% of patients receiving radiation for head and neck cancer. With immunosuppression there is a trend of emergence of rare species. The present study aimed to assess species and colonization of *candida* at different stages of radiation therapy for head and neck cancer.

**Methods:** Oral rinses of thirty cancer patients receiving a six weeks course of radiation therapy for head and neck cancer were taken at two intervals; first at the start of radiation (0 Grays), and second at completion of radiation (60 Grays). The oral rinse was streaked onto a differential media (CHROMagar<sup>®</sup>) plates and incubated at 37°C for 48 hours. Colony forming units (CFU) were counted and species were differentiated. Fifteen healthy controls were compared.

**Results:** The *candida albicans* colony count (CFU/ml) at baseline, 0 Grays radiation ranged from 50 to 1820 CFU/ml in cases and from 0 to 300 CFU/ml in controls. *C. albicans* was seen in all cases (100%) and most of the controls (86.66%). Other species such as *C. Krusei*, *C. parapsilosis*, *C. tropicalis*, and *C. glabrata* were observed with a frequency of 10%, 6.66%, 3.33%, and 3.33% respectively in the cases. However, no species other than *C. albicans* was observed in controls.

**Conclusions:** Immunosuppression of radiation therapy patients led to the development of species other than *Candida albicans*, which is the most prevalent species. Thus it can be inferred that there is emergence of the opportunistic fungal pathogens in patients with immunosuppression.

**Keywords:** candidal colonization; CHROMagar<sup>®</sup>; differential media; immunosuppression.

## INTRODUCTION

In the Indian subcontinent, oral cancer constitutes approximately 40% of all malignant tumors with an incidence of 56,000 cases per year.<sup>1</sup> Irradiation-induced histologic changes (oral mucositis), together with quantitative and qualitative changes in saliva and salivary flow are thought to facilitate yeast infection.<sup>2,3</sup> *Candida albicans*, is the most common commensal yeast of the oral mucosa in healthy individuals<sup>4,5</sup> while

other *candida* species are regarded as opportunists.<sup>6</sup> The incidence of *C. albicans* isolated from the oral cavity has been reported to be 5% (neonates) to about 95% (HIV patients).<sup>6</sup>

Various factors like drugs use endocrinopathy, immunosuppression, infection or advanced neoplasms like head, neck malignancies (especially when treatment is by radiotherapy), organ transplant recipients, poor

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oral hygiene, pregnancy, xerostomia may predispose to candidal colonization.<sup>7,8</sup>

The present study aimed to assess the prevalence of various species as well as number of colonies in normal patients and patients undergoing radiation therapy for head and neck cancer at different stages of radiation. This study highlighted the fact that opportunistic candidal colonization prevails during radiotherapy and that the species may change within the course of the therapy. This is correlated with the clinical condition of the patient who developed oral mucositis.

## METHODS

Thirty patients receiving a six week course of radiation therapy for treatment of head and neck cancer were taken and an informed consent was obtained. Ethical clearance was obtained from the institutional ethics committee (KMC, Mangalore) prior to the commencement of the study. The study included obtaining oral rinses of the radiotherapy patients taken at two intervals during the course of the radiation therapy; one at the first day, i.e at 0 Grays (Gys), and later, upon the completion of radiation schedule, i.e at 60 Grays of radiation. Oral rinse samples were also collected from fifteen controls.

### I. Procedure for candidal colonization:<sup>8</sup>

Cultures for fungi were obtained at the start and end of radiation treatment. Colonization was established on the basis of a positive culture and fungal growth was quantified by counting colony forming units/ml (CFU/ml) on the culture media used. Oral rinse samples were obtained as described by Samaranayake et al<sup>8</sup> with slight modifications. Patients were given 10 ml of sterile normal saline to rinse the mouth for one minute which was emptied in a sterile clean, broad-mouthed container (Stericol). The 10 ml of saliva rinse was centrifuged for 10 minutes, the supernatant discarded and re-suspended with 1ml of saline to the remaining deposit.

Twenty µl of the sample was taken from the one ml saliva solution and streaked onto Hi-chrome agar (CHROMagar<sup>®</sup>) plates and incubated at 37°C for 48 hours.<sup>9</sup> Number of colonies (CFU=n) formed were counted on the agar plate and multiplied with a factor of 50 to get the colonies present in 1ml of patient's sample n x 50 (CFU/ml)

### II. Determination of candidal species:<sup>2</sup>

The oral rinse sample inoculated onto CHROMagar<sup>®</sup> plates was determined according to the colour of the various candidal colonies. Different *candida* species imparted different colors to colonies when incubated in

CHROMagar<sup>®</sup> medium which is a differential medium for candida. The *C. albicans* gave light green colonies with pale edges; *C. parapsilosis* gave pale cream colonies; *C. krusei* produced spreading rose pink colonies with broad, pale edges; *C.tropicalis* produced a purple halo in the agar surrounding dark blue-gray colonies and *C. glabrata* gave dark pink colonies with pale edges.<sup>2, 9</sup>

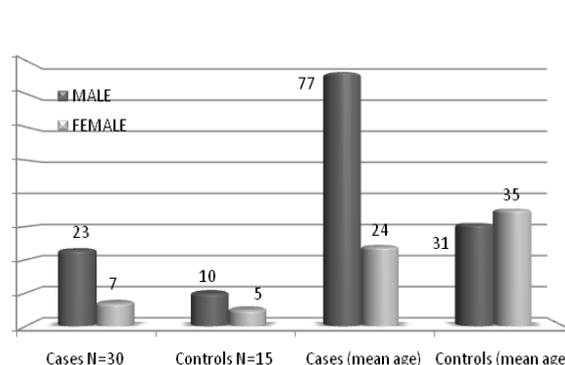
### III. Determination of clinical candidiasis:<sup>10</sup>

A clinical diagnosis of candidiasis was determined on the basis of the clinical presence of pseudomembranous (white) and/or erythematous (red) forms of candidal overgrowth and confirmed based on a positive fungal culture.

The data was uploaded onto an Excel spreadsheet and analysed using SPSS (Statistical Package for Social Sciences version 11). The tests used for the analysis included Wilcoxon's sign rank test, Mann-Whitney U test, Mc Nemars Chi square test and Bonferroni test for multiple comparisons.

## RESULTS

Out of a total of 45 samples (30 cases and 15 controls), 33 (73.33%) were males and 12 (26.66%) were females (Figure1). The tongue was found to be the most common site along with cancers of the larynx, glottis and vocal cord collectively.



**Figure 1. Age and Sex distribution of the cases and controls.**

Assessment of *Candida albicans* colonization between cases and controls by was done by Mann Whitney U test. The *candida albicans* colony count (in CFU/ml) at baseline of 0 Grays radiation ranged from 50 to 1820 CFU/ml; while it ranged from 0 to 300 CFU/ml in controls. The number of CFU of *Candida albicans* increased from baseline (0 Grays) to 60 Grays in all but one patient in whom the value decreased from 120 to 50 CFU/ ml. However, this particular patient

demonstrated an increase in colonization by *C. krusei*. There were controls without any candidal colonization whereas the cases showed a wide variation in the candidal colonization with a median of 425 CFU/ml and an Interquartile range of (487.50) (Table 1).

As determined by Mann Whitney U test, the colonization (in CFU/ml) of *C. albicans* species was observed to significantly higher in cancer patients (596.0 ± 535.81) than in with normal controls (130 ± 103.16) with a “p” value of < 0.001 (0.0000549). The cases showed

a maximum of 1820 CFU/ml and minimum of 50 CFU/ml while the maximum value for controls was 300 CFU/ml. Similarly, candidal colonization (CFU/ml) was found to be significantly increased before radiation, i.e at 0 Grays) (440 ± 491.90) to after radiation of 60 Gys (1286.0± 630.32) in patients with a “P” value of < 0.01 (0.000001889) by Wilcoxons sign rank test (Table 2).

On assessing the frequency of colonization of various candidal species, it was observed that *C. albicans* colonization was seen in all the cases (100%) and in

**Table 1. Assessment of *Candida Albicans* species colonization (in CFU/ml) in cases and controls at 0 Grays of radiation dose (Mann Whitney U test).**

| S No                                | Groups   | N  | Min (CFU/ml) | Max (CFU/ml) | Mean rank | Median (25 <sup>th</sup> , 75 <sup>th</sup> ) | Inter-quartile range | Z      | P value |
|-------------------------------------|----------|----|--------------|--------------|-----------|---|----------------------|--------|---------|
| 1<br>( <i>CAlbicans</i> count at 0) | Cases    | 30 | 50           | 1820         | 28.57     | 425 (200; 687.5)                              | 487.50               | -4.034 | <0.001  |
|                                     | Controls | 15 | 0            | 300          | 11.87     | 100 (50; 200)                                 | 150                  |        |         |

**Table 2. Comparison of *C albicans* colonization (in CFU/ml) before and after radiation radiation dose of 60 Grays (Wilcoxon’s sign rank test).**

| SN | Variable                  | Category | N  | Min | Max  | Median(25 <sup>th</sup> ;75 <sup>th</sup> ) | Mean rank     | Z     | P Value |
|----|---------------------------|----------|----|-----|------|---|---------------|-------|---------|
| 1  | <i>C. albicans</i> colony | 0 Gys    | 45 | 0   | 1820 | 425 (200; 687.5)                            | 1.00;<br>16.0 | -4.76 | <0.001  |
|    |                           | 60 Gys   | 30 | 50  | 2500 | 100 (50; 200)                               |               |       |         |

most of the controls (86.66%) (Figure 3). Other species such as *C. Krusei* (Figure 4), *C. parapsilosis* (Figure 3), *C. tropicalis* (Figure 5), and *C. glabrata* (Figure 5) were observed with a frequency of 10%; 6.66%; 3.33%, and 3.33% respectively in the group of cancer cases. However, there were no other candidal species observed in controls other than *C. albicans* (Table 3).

**Table 3. Cases and controls showing various species of candidal colonization.**

| <i>Candida</i> species      | Cases                  |                         | Controls         |
|-----------------------------|------------------------|-------------------------|------------------|
|                             | 0 Gys No. (percentage) | 60 Gys No. (percentage) | No. (percentage) |
| <i>Candida albicans</i>     | 30 (100)               | 30 (100)                | 13 (86.66)       |
| <i>Candida krusei</i>       | 3 (10)                 | 3 (10)                  | 0                |
| <i>Candida parapsilosis</i> | 2 (6.66)               | 3 (10)                  | 0                |
| <i>Candida tropicalis</i>   | 1 (3.33)               | 1 (3.33)                | 0                |
| <i>Candida glabrata</i>     | 1 (3.33)               | 1 (3.33)                | 0                |

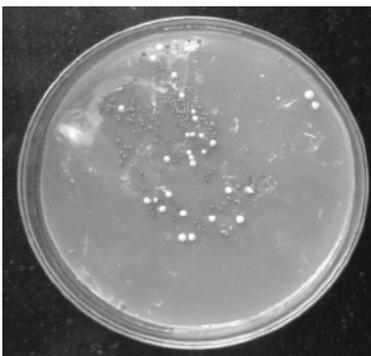
There was a significant increase in clinical candidiasis from baseline (0 Grays) to completion (60 Grays) of radiation therapy with a “p” value of <0.001(0.0000002) by Mc Nemars Chi square test. Clinical candidiasis was absent in the entire control group (0%), and manifested in only 5 of 30 cases (16.66%) at 0 Grays of radiation. However, at 60 Grays of radiation, clinical candidiasis was seen in 28 out of 30 cases (93.33%) (Table 4, Figure 2).

**Table 4. Assessment of clinical candidiasis outcome (McNemar’s Chi square test).**

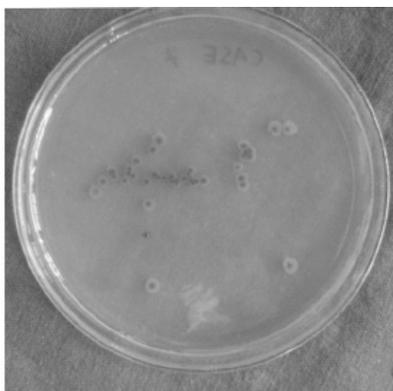
| Clinical candidiasis at 0 Gys | Clinical candidiasis at 60 Gys |        |        | Confidence interval lower limit | Higher limit | P value  |
|-------------------------------|--------------------------------|--------|--------|---------------------------------|--------------|----------|
|                               | Present                        | Absent |        |                                 |              |          |
| Present                       | 5                              | 0      | 16.66% | 53.74%                          | 76.67%       | (<0.001) |
| Absent                        | 23                             | 2      | 3.33%  |                                 |              |          |
|                               | 93.33%                         | 6.7%   | 100%   |                                 |              |          |



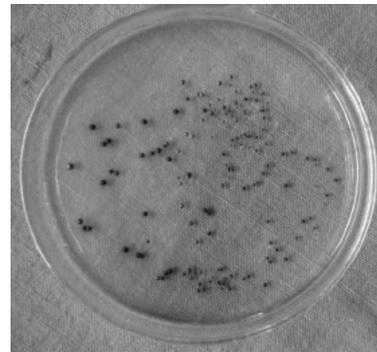
**Figure 2.** Development of Clinical Candidiasis as erythematous lesion and ulcers after radiation of 18 Gys in cases.



**Figure 3.** Mixed colonies of *C. albicans* (light green colonies that was seen in most of the controls and in all cases) and *C. parapsilosis* (pale cream colonies).



**Figure 4.** *C. krusei* produced spreading rose pink colonies with broad, pale edges.



**Figure 5.** Mixed colonies of *C. tropicalis* (purple halo in the agar surrounding dark blue-gray colonies) and *C. glabrata* (dark pink colonies with pale edges).

## DISCUSSION

The immune system of the host is altered not only by tumor but also by the mode of treatment like radiation or chemotherapy.<sup>11</sup> Oral candidiasis is commonly seen in individuals treated with radiation for head and neck cancers.<sup>12</sup>

In the present study, mucosal colonization of *candida* was seen in all the cases (100%) at baseline (0 Grays of radiation); and the colonization increased upon completion of 60 Grays of radiation therapy. This is in accordance with most of the previous studies in which colonization by *candidal* species was seen in upto 93% and candidal infection was seen in 17-29% of patients undergoing radiation therapy for head and neck cancer.<sup>12,13</sup>

The occurrence of candidiasis in radiation therapy patients has been postulated to be a result of local immune suppression as well as disturbances in local factors such as salivary flow and mucositis. The pathogenesis of candidal infections is complex as it encompasses both fungal and host factors<sup>14</sup> and may be influenced by adherence between fungi and epithelial cells.<sup>5</sup>The presence of a highly irregular surface as present in a fungating growth of carcinoma may lend itself to harbor greater numbers and more candidal species. This may account for the absence of clinical lesions associated with candidiasis in the healthy controls in this study despite the presence of candidal colonization.

Radiation therapy may accentuate candidal colonization in the following ways:

1. Compromised salivary function secondary to radiation-induced destruction of glandular tissue thereby causing quantitative and qualitative changes in saliva.<sup>13</sup>
2. Irradiation-induced tissue damage leading to oral mucositis creates a scaffold for fungal adherence.<sup>12</sup> The proteinase that is induced at an early stage probably facilitates epidermal invasion by *Candida*.<sup>15,16</sup>

The increased candidal colony count from baseline to completion of therapy could be due to marked immunosuppression and occurrence of mucositis that created a rough mucosal surface in later stages of radiotherapy in our study. All species of *Candida* increased greatly in number (CFU/ml) in the present study. Ramirez-Amador *et al* in their study on patients undergoing radiotherapy for oral and pharyngeal cancers have shown that the number of heavy colonies (i.e., >500 CFU/ml) increased from 17% at baseline to 45% upon completion of radiotherapy.<sup>5</sup> The present study showed a significantly lower colonization (0-300 CFU/ml) and no other species apart from *C. albicans* in healthy controls.

Clinical candidiasis is directly proportional to the number of colonies. The present study showed that in the baseline of radiation therapy, clinical candidiasis was present only in those patients having colony counts greater than 500 CFU/ml. However, clinical candidiasis was not dependant on the sub-type of organism isolated. On clinical examination, most of the patients had accompanying symptoms of burning sensation and/ or Xerostomia which was similar to that found in other studies.<sup>5</sup> As none of the control group in our study had the presence of clinical infection despite the colonization, it supports the fact that *Candida* is a commensal pathogen and the most prevalent fungal species as was determined by various studies.<sup>9,12,13</sup>

The presence of candidiasis in the present study was determined by the clinical presence of pseudomembranous (white) and/or erythematous (red) forms of candidal overgrowth and the diagnosis was confirmed on the basis of a positive fungal culture as followed by Ramirez-Amador *et al*.<sup>5</sup> Most of the lesions started as pseudomembranous candidiasis (PC) and were followed by same or erythematous candidiasis (EC) lesions in many patients in the later stages. Pindborg and Nielsen have discussed whether pseudomembranous candidiasis (PC) is a precursor of the erythematous variant or *vice versa*. Various studies have shown that most of the erythematous lesions were initially pseudomembranous, but the thick covering would have been removed by oral dynamics like tongue and

muscular movements.<sup>15,16</sup> Some stated that PC is an expression of a defect in the local immune response of the host, whereas EC is accompanied by activation of a partially reactive defense mechanism and may represent a clinical expression in response to candidal antigens.<sup>15,16</sup>

In the present study, we observed *C. Krusei*, *C. parapsilosis*, *C. tropicalis*, and *C. glabrata* among the non candidal species. The other studies showed *C. tropicalis* and *C. parapsilosis* to be the non albicans species isolated commonly in similar groups of patients.<sup>12</sup> To justify the predominance of *Candida albicans* over other candidal species, Kirkpatrick *et al* emphasized its competitive growth advantage over other species when grown together in planktonic conditions promoting bio-film formation.<sup>5</sup> The *C. albicans* secretes enzymes such as phospholipases and proteinases that impart virulence to this strain. The proteinases remain expressed on the surface of intracellular fungal elements thus preventing normal antimicrobial activity of phagocytes.<sup>16</sup>

## CONCLUSIONS

Immunosuppression of the cancer patients led to the development of infection with *Candida albicans* which was the most common opportunistic fungal organism of the oral cavity. When radiation therapy was employed in these patients, there was emergence of different candida species other than *Candida albicans* in most of the cases after the completion of the therapy. Thus, there was emergence of the opportunistic fungal pathogens in patients with immunosuppression.

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