Research Article

Prevalence of Candida Species in the Oral Cavity of Oral Cancer Patients Undergoing Radiotherapy

Barsha Bajracharya[1], **Subrata Bhattacharyya**[2], **Pratibha Poudel**[3]

[1]Department of Dental Surgery, Nepalese Army Institute of Health Sciences, Bhandarkhal, Kathmandu, Nepal; [2]Department of Oral Pathology, Kantipur Dental College, Basundhara, Kathmandu, Nepal; [3]Department of Oral Pathology, Kathmandu University School of Medical Sciences, Dhulikhel, Kathmandu

Abstract

Objective: To evaluate prevalence of various species of candida in the oral cavity of oral cancer patients undergoing head and neck radiotherapy.

Method: 60 patients of oral cancer undergoing radiotherapy who have received at least 40 grays of radiation and 60 non-cancer controls were included in the study. Oral swab was taken and cultured on Sabouraud's Dextrose Agar media. In the cases positive for candida, three tests were done for species identification: Germ tube test, growth in Chromogenic agar media and carbohydrate assimilation test.

Results: The most prevalent species isolated was Candida albicans (61.1%). The most common non-albicans species was Candida tropicalis (20.4%). Other two species isolated were Candida krusei (11.1%) and Candida glabrata (7.4%). However, in the control group, Candida albicans was the only species isolated.

Conclusion: Although C. albicans is the most prevalent species isolated in oral cancer patients undergoing radiotherapy, non-albicans species have also been emerging as opportunistic pathogens.

Received | 26-04-2018: **Accepted** | 10-06-2019

Corresponding Author | Dr. Barsha Bajracharya, Department of Dental Surgery, Nepalese Army Institute of Health Sciences, Bhandarkhal, Kathmandu, Nepal. **Email:** dr.barsha.bajracharya@gmail.com

Keywords | candida species; oral cancer; radiotherapy.

Introduction

Candida is a common commensal of the oral cavity. In radiation induced hyposalivation, colony overgrowth in combination with the adherence of Candida to the epithelial cells leads to transformation of Candida from the state of commensal to that of a pathogen. The prevalence of oral fungal infection in oral cancer patients receiving radiotherapy is 7.5% before radiotherapy, 39.1% during radiotherapy and 32.6% after completion of radiotherapy.[1,2] The most commonly encountered species of candida in the oral cavity is C. albicans. However, increase in incidence of non-albicans species like C. glabrata, C. tropicalis, C. parapsilosis, C. guilliermondii, C. krusei and C. dubliniensis has been reported in the oral cavity of these patients.[3] Both albicans and non-albicans species of candida cause disease of the same spectrum but they differ in severity, virulence and antifungal susceptibility. Hence due to this changing pattern in the species of candida, studies involving identification of different species of candida can help in improvement of treatment strategies and ultimately better management of these patients.[4] The aim of this study was to assess the prevalence of various species of candida in oral cancer patients undergoing radiotherapy.

Method

60 oral cancer patients who had received at least 40

gray of radiation were included as cases. 60 age and sex matched non-cancer individuals were taken as control. Patients under antibiotics, diabetic patients and patients using intraoral prosthesis were excluded. After taking history, oral examination was done to check the clinical presence of oral candidiasis. The presence of pseudomembranous (white) and/or erythematous (red) forms were considered positive for clinical candidiais.[5] Direct microscopic examination for candidal hyphae was done by taking specimen from the oral cavity with the wooden spatula and making smear on the slide. It was heat fixed and gram stained. Presence of gram-positive candidal hyphae/pseudohyphae was considered as presence of pathogenic form of candida. Hence, cases that showed oral candidiasis clinically and presence of hyphae microbiologically were considered positive for candidal infection.

Candida colonization was determined based on positive culture on Sabouraud's Dextrose Agar (SDA) media. Oral swab samples were collected by asking the patients to rinse the mouth with water and then firmly rubbing a sterile swab on the buccal mucosa, hard palate, floor of the mouth, tongue and gingiva. The swab was then cultured on SDA with 50mg/ml chloramphenicol to prevent bacterial growth. The plates were incubated at 37ºC for up to one week. Creamy white, smooth pasty colonies and opaque colonies with yeasty odour were reported as positive growth for Candida. In the cases of positive growth for candida, tests for species identification were done. Determination of Candida species was done by three methods: germ tube test, culture in chromogenic agar media and carbohydrate assimilation test (table 1).

First, Germ tube test was performed by taking 0.5 ml of pooled human serum into a test tube and emulsifying a colony of yeast was into it to obtain faintly turbid suspension. It was incubated at 37°C in incubator for 2 to 3 hrs. Then, a drop of serum was transferred to a slide using a sterile Pasteur pipette. Microscopic examination of the mount was done to check if the germ tubes had formed. Germ tubes arise from the yeast cell directly. Cases with tubes having parallel walls without constriction at the point of their origin, without nucleus and having three to four times the length and half the width of the yeast cell were considered positive for germ tube. Cases without hyphal extension or with a short hyphal extension and constriction at the point of their origin were considered negative for germ tube Those that showed germ tube were considered as C. albicans or C. dubliniensis.[6]

The isolates from the SDA medium were then inoculated on Chromogenic agar medium and incubated at 37ºC for 48 hours. Growth on the Chromogenic agar was observed within 24 to 48 hours. Only pigmented colonies were considered for species identification. Color of the colonies were then noted and different species of candida were identified by comparing it with the standard growth color provided by the manufacturer of chromogenic agar and by referring to different literatures. [2]

Finally, species of candida was confirmed by carbohydrate assimilation test. A lawn culture of the pre-incubated broth was prepared on the Yeast Nitrogen Base Agar plate. Various sugar disks (Glucose, Sucrose, Maltose, Lactose, Xylose, Melibiose,

Table 1: *Candida species identification chart for medically important Candida species*

Species	Gram stain (Hyphae)	Growth on SDA a37^{0}C	Germ Tube Test	Chromogenic agar media	Assimilation of:								
					Glucose	Maltose	Sucrose	Lactose	Melibiose	Raffinose	Cellobiose	Trehalose	Xylose
C. albicans	+	+	+	Light green	+	+	+	-	-	-	-	+	+
C. dubliniensis	+	+	+	Green	+	+	+	-	-	-	-	+	+
C. glabrata	-	+	-	Pale	+	+	-	-	-	-	-	+	-
C. guilliermondii	+	+	-	Pale pink, purple	+	+	+	-	+	+	+	+	+
C. kefyr	+	+	-	Pink, Purple	+	-	+	+	-	+	+	-	+
C. krusei	+	+	-	Purple fuzzy	+	-	-	-	-	-	-	-	-
C. parapsilosis	+	+	-	White, pale pink	+	+	+	-	-	-	-	+	+
C. tropicalis	+	+	-	Steel Blue	+	+	+	-	-	-	+	+	+

Trehalose, Raffinose and Cellobiose) were placed on the plate and incubated for 24-72 hours. The presence of growth around the disc was considered as positive for that particular carbohydrate. Growth around glucose disc was recorded as a positive control as all candida species assimilate glucose[7]. Results were noted and tabulated. Statistical analysis was performed using SPSS version 23. The level of significance was set at 5%.

Results

Demographic and clinical details of the cases are given in table 2. In this study, 66.6% of oral cancer patients and 8.3% of non-cancer controls had clinical oral candidiasis (Fig.1). All the cases with clinical oral candidiasis also showed candidal hyphae on direct microscopic examination by gram staining. They were interpreted as having oral candida infection. Culture on SDA media showed Candidal growth (Fig. 2) in 90% of cancer patients and 20% of non-cancer controls. They were interpreted as having oral candida colonization. For identification of species, germ tube test was carried out first. 61.1% of cases and all the controls that showed candidal growth in SDA gave positive Germ tube test (Fig.3). These were identified as either C. albicans or C. dubliniensis. Those that did not produce germ tube were considered as rest of non-albicans species.

Table 2: *Clinical and demographic characteristics of the cases*

Variables	Category	n (%)
Age (yrs)	• Mean ± sd	57.70±12.48
	• Range	25 to 85
Gender	• Male	44 (73.3%)
	• Female	16 (26.7%)
Tumor site	• Tongue	19
	• Buccal vestibule	15
	• Floor of mouth	7
	• Labial vestibule	4
	• Retromolar Trigone	4
	• Others	11
TNM stage	• II	11 (18.3%)
	• III	23 (38.3%)
	• IVa	21 (35.0%)
	• IVb	5 (08.3%)
Treatment received	• Radiotherapy and chemotherapy	21 (35%)
	• Surgery, Radiotherapy and chemotherapy	39 (65%)
Radiation dose (Gy)	• Mean ± sd	55.26±9.229
	• Range	40 to 76

Further, for species identification, colorful growths in chromogenic agar (CHROMagar) media were evaluated (Fig.4). Those with light green colonies were identified as C. albicans (61.1% of cases that showed growth in SDA and all the controls). Since C. dubliniensis besides producing germ tube also develop colonies of the color similar to that of C. albicans, it was important to differentiate C. albicans from C. dubliniensis. According to Milan and Zaror, these two species produce different intensity of color in CHROMagar, C. albicans colonies are green-blue pale and C. dubliniensis colonies are dark-green.[8] Since all the growth which showed positive germ tube test produced green-blue pale color and matched that of the standard color provide by the manufacturer and other published studies, they were identified as C. albicans. 20.3% of cases with positive growth on SDA showed blue colored colony and was identified as C. tropicalis. 11.1% of cases showed purple fuzzy growth and was identified as C. Krusei. 7.4% of growth showed cream-colored growth and was identified as C. glabrata.

The species identification was finally confirmed by carbohydrate assimilation test (Fig 5). All the cases and controls that were identified as C. albicans in CHROMagar, assimilated glucose, maltose, sucrose, trehalose and xylose and hence were confirmed as C. albicans. Cases that were identified as C. tropicalis assimilated glucose, maltose, sucrose, cellobiose, trehalose and xylose, and hence were confirmed as C. tropicalis. Those, which assimilated glucose, maltose and trehalose, were confirmed as C. glabrata (7.4%). 11.1% of cases identified as C. Krusei assimilated only glucose and hence were confirmed as C. Krusei.

Hence, based on these three tests, among the cases that showed candida colonization, the most prevalent species of candida was Candida albicans (61.1%). The most common non-albicans species was Candida tropicalis (20.4%). Other two species isolated were Candida krusei (11.1%) and Candida glabrata (7.4%). However, in the control group, only Candida albicans was isolated. Multiple species in the same case were not isolated. (Table 4)

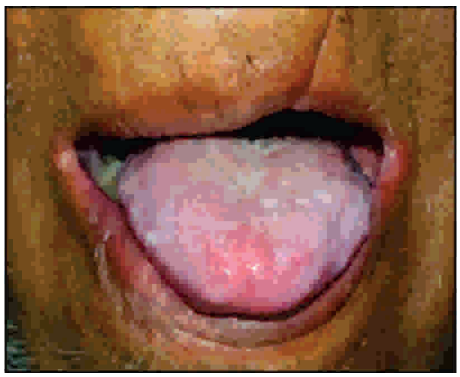

Figure 1. Pseudomembranous candidiasis

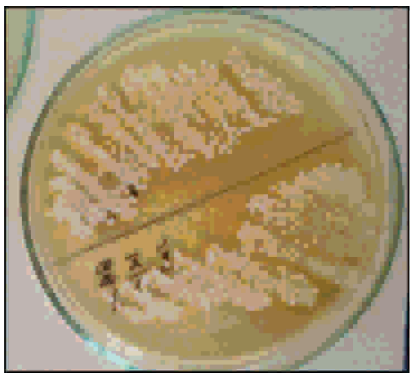

Figure 2. Candidal growth on SDA

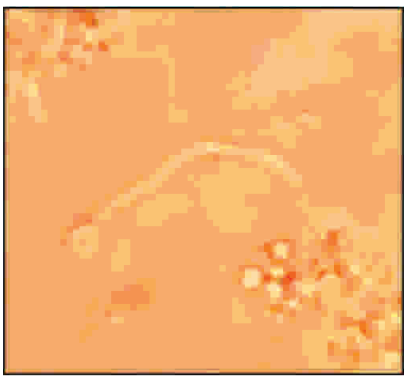

Figure 3. Germ tube

(a) (b) (c) (d)

Figure 4. Growth in Chromogenic Agar Media: a C.albicans, b. C. tropicalis, c.C. Krusei, d. C. glabrata

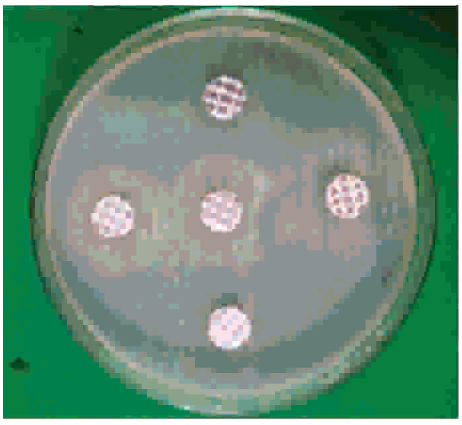

Figure. 5 Carbohydrate assimilation test showing growth around carbohydrate discs

Table 3: *Comparison of Candida species among the cancer patients and non-cancer controls*

Candida species	Study Participants with oral candida colonization		P value
	Cancer patients (n = 54)	Non-cancer Controls (n =12)	
C. albicans	33 (73.3%)	12 (26.7%)	0.077
C. tropicalis	11 (100%)	0	----
C. krusei	06 (100%)	0	----
C. glabrata	04 (100%)	0	----

Discussion

Oral candidiasis is an opportunistic infection commonly seen in oral cancer patients undergoing radiotherapy. Studies have reported incidence of oral candidiasis to be ranging from 7% to 52% in these patients.[9] Colonization is defined as the presence of yeast cells in the oral cavity with or without clinical signs and symptoms. On the other hand, candidal infection or oral candidiasis is defined as the demonstration of gram positive hyphae/ pseudohyphae and yeast cells microbiologically along with clinical signs and symptoms. Patients usually progress from asymptomatic colonization stage to infection. Candida infection in patients with malignancy can progress to invasion into deeper tissues if not treated promptly.[10]

In our study, 66.6% of the cancer cases and 8.3% of the non-cancer controls showed clinical candidiasis. Presence of candidiasis in controls can be because most of the controls were in and over fifth decade of life. Age related changes in immunity and oral mucosa (e.g.: coated tongue, atrophic glossitis, fissured tongue etc.) predisposes old people to candidiasis.[11] Many authors have discussed whether pseudomembranous variant is a precursor of the erythematous candidiasis 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.[10] 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. Pathogenesis of candidal infection encompasses both fungal and host factors and is influenced by adherence of candida to the epithelial cells.[12] According to Shrestha et al., 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.[5]

In this study, species of candida was confirmed by using three methods: germ tube test, chromogenic agar media and carbohydrate assimilation test. Among the cases, C. albicans was the most prevalent species (33, 61.1%). Among the non-albicans species, C. tropicalis (11, 20.4%) was most commonly isolated, followed by C. Krusei (6, 11.1%) and C. glabrata (4, 7.4%). This finding is similar to that of study conducted by Shrestha et al. in India (2014)[5], which also reported C. albicans as most commonly isolated species in the oral cavity of head and neck radiotherapy patients and the only species isolated in controls. Among cases, the most commonly isolated non-albicans species in their study were C. krusei, followed by C. parapsilosis, C. tropicalis and C. glabrata. According to their study, there was emergence of non-albicans species in most of the cases after the completion of the therapy.

Redding et al. in their study justified the predominance of C. albicans over other candidal species by emphasizing its competitive growth advantage over other species when grown together in planktonic conditions promoting biofilm formation.[13] Studies have shown that 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.[14]

Manas et al. in their study also reported that the most prevalent species of candida colonizing oral cavity was C. albicans (69%) and non-albicans species were isolated in 31% of patients. They gave two explanations for the emergence of non-albicans species in oral candidiasis. Firstly, use of azolic drugs for antifungal prophylaxis since some non-albicans are less sensitive to azoles. Secondly, non-albicans species of candida may have been present since past but it may not have been possible to detect them until recently due to invention of newer sensitive methods allowing better species identification.[15]

Belazi et al.[16] in a similar study performed antifungal susceptibility test in those cases with confirmed candidiasis. Their study showed that 8/23 C. albicans, 2/2 C. krusei and 2/3 C. glabrata isolates were resistant to fluconazole and susceptible to itraconazole. All of the isolates showed very low voriconazole MICs. Their study supported the requirement for regular in vitro antifungal susceptibility testing of the isolates in the cases with confirmed candidiasis. These tests can detect resistance to the azoles that are used routinely for the management of candidiasis. However, in our study such antifungal susceptibility test could not be performed, and hence is a drawback of this study.

Conclusion

The present study suggests that there is a definitive association between head and neck radiotherapy and oral candidiasis. Though C. albicans is the most common species isolated in these patients, non-albicans species have also been emerging as opportunistic pathogens. As non-albicans species have different epidemiology, virulence and antifungal susceptibility, species identification can help in better treatment strategy and consequently a good control over the disease.

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