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Original Article

Comparison of oral candidiasis characteristics in head-and-neck cancer patients before and during radiotherapy

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ABSTRACT

Background: Patients undergoing head-and-neck radiotherapy are susceptible to *Candida* colonization and infection. This study aimed to identify oral *Candida* species type (ST), colony count (CC), and oropharyngeal candidiasis (OPC) in head-and-neck cancer patients, undergoing radiotherapy, before and 2 weeks after radiation.

Materials and Methods: In this quasi-experimental study, head-and-neck cancer patients undergoing radiotherapy (up to 6000 cGy) were recruited. Samples were taken before and 2 weeks after radiation therapy (RT). CC was assigned using Sabouraud dextrose agar culture medium and morphological studies were performed to confirm OPC. For identification, polymerase chain reaction–restriction fragment length polymorphism was performed. Data were analyzed using Chi-square-test and kappa coefficient. $P < 0.05$ was considered statistically significant.

Results: Twenty-one of 33 patients were *Candida* positive. The detected fungal species included *Candida albicans* (60%), *Candida tropicalis* (22%), *Candida glabrata* (9%), and other species (9%). Following RT, OPC and CC changed significantly ($P = 0.003$ and $P = 0.001$, respectively), whereas ST did not significantly change ($P = 0.081$). Two new species (*Candida krusei* and *Candida parapsilosis*) were detected after the intervention. The OPC, CC, and ST changes after RT were not significantly related to malignancy site or radiation dose ($P > 0.05$).

Conclusion: The present study showed that OPC, CC, and ST were not related to the malignancy site. Following RT, OPC and CC changed significantly, while ST showed no significant change. The radiation dose and malignancy site had no effects on the OPC, CC, or ST alterations following RT.

Key Words: *Candida,* head-and-neck neoplasms, radiotherapy

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INTRODUCTION

Oropharyngeal candidiasis (OPC) is the most common fungal infection in cancer patients. Patients receiving head-and-neck radiotherapy are more susceptible to *Candida* colonization and infection. The prevalence of OPC is highly variable among patients, ranging from 11% to 55%.[1-4] Therapeutic radiation to the head and neck can be followed by both acute and chronic oral complications, such as radiation-induced mucositis and xerostomia. These side effects usually occur 1–2 weeks after the initiation of treatment and trigger *Candida* overgrowth.[5,6]

The most frequent *Candida* species causing OPC in head-and-neck cancer patients undergoing radiotherapy is *Candida albicans*.[7,8] However, other species of the genus *Candida* such as *Candida parapsilosis*, *Candida krusei*, *Candida lusitaniae, Candida stellatoidea*, *Candida guilliermondii*, and *Candida dubliniensis* have been shown to increase the incidence of fungal infections.[9] It is known that geographic location influences the species distribution. For instance, while *Candida glabrata* is the predominant non-*C. albicans* species in North America, *Candida tropicalis* is the predominant species in Brazil.[10] Therefore, it seems reasonable to identify species in different areas.

The present study aimed to identify the oral *Candida* species type (ST), colony count (CC), and OPC in head-and-neck cancer patients undergoing radiotherapy before and 2 weeks after radiation.

MATERIALS AND METHODS

This was a quasi-experimental study.

Sampling

Head-and-neck cancer patients undergoing radiotherapy at Seyed-Al-Shohada Hospital, Isfahan University of Medical Sciences, Isfahan, Iran, participated in this study. The inclusion criteria were as follows:

1. A minimum total radiation dose of 6000 cGy and a minimum daily radiation dose of 180 cGy
2. No history of chemotherapy before radiation therapy (RT)
3. No history of antifungal or antibiotic therapy in the past 3 months.

The exclusion criteria were smoking, diabetes mellitus, immunodeficiency, mental retardation, and wearing an oral prosthesis. Two samples were taken from each patient (before RT and 2 weeks after RT). Two saline moisturized swabs were contaminated with the mucosa of the oral cavity.

Laboratory procedure

The first swab was drawn on a slab and checked for the presence of *Candida* species through Giemsa staining. The second swab was cultured on Sabouraud dextrose agar, containing chloramphenicol 0.5% for CC, and morphological studies were performed to confirm OPC. A second culture of colonies from the former culture was prepared for identifying *Candida* species. For identification, polymerase chain reaction–restriction fragment length polymorphism was performed using internal transcribed spacers (ITS1 and ITS4) and MspI restriction enzyme.

Statistical analysis

Head-and-neck cancers were divided into intraoral (oral cavity and pharynx) and extraoral (larynx, neck, thyroid, ear, and carotid body) malignancy groups. *Candida* species were categorized as *C. albicans* and non-*C. albicans*. The CC was also categorized into three groups: 0 CFU; 1–1000 CFU; and >1000 CFU. The parotid radiation dose was divided into under 2000cGy and above 2000cGy. Chi-square test was used for analyzing the relationship between the malignancy type and OPC, CC, and ST before and after RT and for assessing the relationship between radiation dose and OPC, CC, and ST. Finally, the kappa coefficient was measured to compare OPC, CC, and ST before and after RT. $P < 0.05$ was considered statistically significant.

RESULTS

Overall, 57% of the patients had extraoral malignancies, while 43% were diagnosed with intraoral malignancies. The total parotid radiation dose ranged from 4.66 cGy to 4104.79 cGy, with a mean of 1857.39 cGy. Table 1 shows *Candida* detection and ST among patients. Figure 1 shows the frequency of species in candida positive patients before and two weeks after radiation therapy.

The CC and ST classified by malignancy type (extra/intraoral) are shown in Table 2.

The relationship between OPC, CC, and ST alterations and radiation dosage is shown in Table 3.

The OPC was negative in two patients before the intervention, while it became positive during RT. In contrast, two OPC-positive patients became negative following RT. The CC was 0 in 11 patients, 1–1000 in ten

Table 1: Species-wise number of *Candida* positive patients before and 2 weeks after radiation

Species	Number before, *n* (%)	Number following 2 weeks of radiation, *n* (%)
C. albicans	12 (36)	11 (40)
C. tropicalis	3 (10)	3 (10)
C. glabrata	2 (6)	1 (4)
C. parapsilosis	0	1 (4)
C. albicans/C. tropicalis	2 (6)	0
C. tropicalis/C. glabrata	0	1 (4)
C. albicans/C. krusei	0	1 (4)
Other	2 (6)	1 (4)
Negative	12 (36)	8 (30)
*Total	33	27

*Six patients (18%) expired during 2 weeks of the study. *C. albicans*: *Candida albicans, C. tropicalis: Candida tropicalis, C. glabrata: Candida glabrata, C. parapsilosis: Candida parapsilosis, C. krusei: Candida krusei*

Table 2: The relationship between oropharyngeal candidiasis, colony count, and species-type alterations with malignancy type

	Oral malignancy	Extraoral malignancy	*P*
OPC (%)			
Positive	21.4	57	0.97
Negative	78.6	43	
CC (%)			
0	50	31.6	0.49
1-1000	28.6	47.3	
>1000	21.4	21.1	
ST (%)			
C. albicans	28.6	41.2	0.26
Non-albicans	21.4	31.6	
Mixed yaest*	50	26.3	
OPC alteration			
Yes	16.7	13.3	1.00
No	83.3	86.7	
CC alteration			
Yes	33.3	40.00	1.00
No	66.7	60.00	
ST alteration			
Yes	50.00	53.3	0.86
No	50.00	46.7	

C. albicans: Candida albicans, OPC: Oropharyngeal candidiasis, CC: Colony count, ST: Species type

patients, and >1000 in six patients before RT. Following RT, five patients showed an increase in CC from 0 to 1–1000. In patients with 1–1000 CC, two showed an increase to CC >1000, while two others showed a decrease to zero. Only one patient in the CC >1000 group showed decreased CC to 1–1000 after RT [Table 4].

DISCUSSION

Twenty-one of 33 patients were *Candida*-positive before treatment. Fungal species, CC, and OPC included *C. albicans* (60%), *C. tropicalis* (22%), *C. glabrata* (9%), and other species (9%). Suryawanshi *et al*. and Kurnatowski *et al*. reported the same results (*C. albicans* 60%). In Suryawanshi *et al*.'s study, three and five different *Candida* species were detected before and after RT, respectively.[11] Furthermore, Kurnatowski *et al*. isolated six and eight different species before and after RT, respectively.[12] *C. krusei* and *C. parapsilosis* were detected as new species in our study, while *C. lusitaniae* and *C. guilliermondii* were their newly detected species. The PCR method was applied for species identification in the present study rather than only morphological and biomedical analyses.

In the present study, 21.4% of the patients with intraoral malignancies were OPC positive. Wisniewski *et al*. reported that 17 of 30 patients with oral cancer were *Candida* positive.[13] Čanković and Bokor-Bratić found that 30% of oral cancer patients were positive for *Candida*, 55% of whom had species other than *C. albicans*.[14] In our study, 57% of the patients with extraoral malignancies were OPC positive. Similarly, Krajewska-Kułak *et al*. revealed fungi in the oral cavity of 55.9% of patients with cancer versus 24.4% of healthy individuals.[15] In the present study, the difference in terms of CC, ST, and OPC between the two malignancy types (extra/intraoral) was not significant ($P = 0.49$, $P = 0.26$, and $P = 0.97$, respectively).

The risk factors for *Candida* carriage and OPC include poor oral hygiene, smoking, wearing dentures, and xerostomia.[16] Besides, OPC is common among patients with prolonged severe diseases, such as diabetes, organ transplantation, and a history of chemotherapy.[17-19] The carriage of *Candida* in the oral cavity of cancer patients is much higher than of healthy individuals, and non-*albicans* species play an important role in their infection. These species are more prevalent in cancer and critically ill patients.[20,21] Although the main cause remains controversial, alterations in the oral mucosal tissue, saliva quality/quantity changes, and underlying immunodeficiencies may be involved.[22]

Importantly, the current study excluded patients with local risk factors for candidiasis, including smoking, use of dentures, history of topical drug use, diabetes, and history of antibiotic therapy/chemotherapy to determine the effect of underlying cancer on OPC, CC, and ST. The site of tumor affected OPC, CC, or

Table 3: The relationship between oropharyngeal candidiasis, colony count, and species-type alterations and radiation dosage

	<2000 cGy	>2000 cGy	*P*
OPC alteration			
Yes	21.4	7.7	0.59
No	78.6	92.3	
CC alteration			
Yes	35.7	38.5	1.00
No	64.3	61.5	
ST alteration			
Yes	57.1	46.2	0.70
No	42.9	53.8	

OPC: Oropharyngeal candidiasis, CC: Colony count, ST: Species type

Table 4: The relationship between oropharyngeal candidiasis, colony count, and species type before and after radiation

	Yes (%)	No (%)	*P*
OPC alteration	15	85	0.003
CC alteration	37	63	0.001
ST alteration	52	48	0.081

OPC: Oropharyngeal candidiasis, CC: Colony count, ST: Species type

ST neither before treatment nor following RT. Poor oral hygiene related to lifestyle modification and underlying immunodeficiencies might explain this finding regardless of the cancer site.

Similar to our study, Lou *et al*. calculated the exact planning dose of parotid gland radiation. Besides, they focused on the percentage of the parotid gland volume, which received different radiation doses. They concluded that neither a low radiation dose (<20 Gy) to a high volume of the parotid gland (>60%) nor a high radiation dose (40 Gy) to a low volume of the parotid gland (<20%) was significantly related to xerostomia. Xerostomia occurred when at least 50% of the parotid tissue received more than 20 Gy radiation doses simultaneously.[23] Overall, xerostomia is a known predisposing factor for OPC, *Candida* species change, and CC increase.[24]

The present study did not show any significant relationship between OPC, CC, and ST alterations and radiation dose. It seems that besides beam quantity, the volume of radiated gland also plays an important role in xerostomia and candidiasis. In this regard, Nishii *et al*. showed that among several factors, severe mucositis, low levels of serum leukocytes, and tumor size were significantly related to candidiasis. In line with our results, radiotherapy and total radiation dose had no effect on *Candida* occurrence.[25]

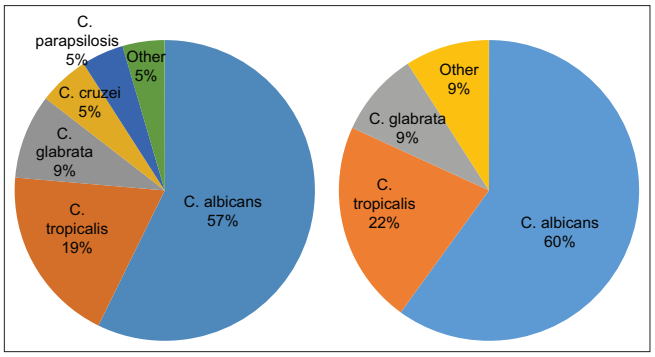

Figure 1: The frequency of species in *Candida*-positive patients before and 2 weeks after radiation therapy.

The present study showed that after 2 weeks of RT, OPC and CC changed significantly (P = 0.003 and P = 0.001, respectively), whereas ST did not significantly change (P = 0.081). Similarly, Lalla *et al*. found that following treatment, OPC increased from 7.5% to 39.1%. It should be noted that their patients underwent combined chemotherapy/RT in their study, whereas our patients received RT alone.[1]

Moreover, Kurnatowski *et al*., Dwornika *et al*., and Ptyko *et al*. reported an increase in *Candida*-positive patients following RT. In contrast to our study, they only reported positive/negative detection or growth of *Candida*, whereas our study focused on the detailed properties of candidiasis, including CC, ST, and OPC.[12,26,27] In this regard, Kurnatowski *et al*. showed an increase in *Candida*-positive patients from 66.2% to 80% following 3 weeks of RT.[12] Ptyko *et al*. also reported an increase of 22% in *Candida* detection after 2 weeks of RT from 46.3% to 68.3%.[26] Moreover, Dwornika *et al*. reported the growth of *Candida* after 2 to 3 weeks of RT at two sites of the oral mucosa (cheeks and tongue). The growth of *Candida* was observed in 5.6% of the cases in both sites before RT, while it changed to 38.9% and 19.7% following RT, respectively.[27]

Consistent with our findings, Kurnatowski *et al*. found *C. albicans* as the most common *Candida* species both before and after RT.[12] In contrast, Nucci *et al*. found *C. glabrata* to be the predominant species following RT.[28] Unlike other studies, ST was statistically analyzed in the present study; according to the results, although raw data showed an increased diversity following RT, it was not statistically significant. The combination of oral and biological conditions in a patient leads to the evolution or determination of OPC. The responsible factors include the quantity and quality of saliva, xerostomia, reduced oral rinse,

mucositis, and inability to practice oral hygiene correctly.[22,29]

In general, alterations in the saliva and mucositis begin in the 1st week of RT, resulting in oral biological changes and, therefore, the increased risk of OPC.[30] On the other hand, Ramla *et al*. found that radiotherapy could reduce fungal adherence to epithelial cells. In other words, radiotherapy might cause raw and tender mucosa in the oral cavity and eliminate the suitable conditions for many fungal cells to penetrate into the host tissue by their germ tubes *in vivo*;[22] this controversial finding may explain variations in OPC and ST.

This study had some limitations. First, since the exclusion criteria were strictly observed, the sample size was limited in the present study. Second, an estimated dose rather than an accurate radiation dose to the gland was measured in this study, regardless of the tissue volume. It is suggested that the chronic side effects of radiation be studied on more participants using adjuvant beam calculation techniques in the future.

CONCLUSION

The present study showed that OPC, CC, and ST were not related to the malignancy site. Following RT, OPC and CC changed significantly, while ST showed no significant changes. The radiation dose and malignancy site had no effects on OPC, CC, or ST alterations following RT.

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Conflicts of interest
The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or non-financial in this article.

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