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An *in vitro* study of isolation of candidal strains in oral squamous cell carcinoma patients undergoing radiation therapy and quantitative analysis of the various strains using CHROMagar

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Abstract

Background: Patients undergoing radiation therapy for oral squamous cell carcinoma (OSCC) have an increased risk of developing oral candidiasis. Radiation-induced hyposalivation is thought to be a major predisposing factor for it. Radiation therapy for 2–3 weeks leads to increase in the number of candidal species in the oral cavity, eventually leading to oral candidiasis. **Objective:** To evaluate and correlate the prevalence of candidal species in the oral cavity of OSCC patients and to compare with radiation dosage at 3rd and 6th week following radiotherapy. **Materials and Methods:** This study includes fifty patients undergoing radiation therapy for OSCC. Patients were examined at 3rd and 6th week following radiation therapy, and smear samples were obtained from lesional sites and the radiation dose was recorded. Smear samples were cultured on CHROMagar, and identification of various candidal species was done on the basis of colony color and their morphology. **Results:** The present study isolated four types of candidal species, namely, *Candida albicans, Candida glabrata, Candida tropicalis* and *Candida krusei*. All the organisms, except *C. krusei*, showed a significant increase from 3rd to 6th week of radiotherapy whereas *C. glabrata* and *C. tropicalis* showed a sudden exponential increase. Total radiation dosage did not show any correlation to candidal colonization at 3rd and 6th week following radiotherapy. **Conclusion:** OSCC patients undergoing radiotherapy show an increase in candidal colonization which is independent of radiation dosage and may be related to other factors. However, such findings should be further evaluated using a larger sample size.

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Full Text

Introduction

Candida is a part of the normal oral flora. The balance between candidal colonization and mucosal candidiasis depends on the effectiveness of the host defenses which can be affected by radiation therapy besides other factors.

Patients with head and neck cancer are subjected to high doses of radiotherapy in the oral cavity, which cause many adverse reactions. Typical early side effects are mucositis and xerostomia accompanied by oral colonization and infection with Candida. Compromised salivary function secondary to destruction of glandular tissue by radiation is thought to be a major factor leading to candidal infection. Among the candidal infections, Candida albicans is the most predominant organism to be isolated. Recently, other candidal species have also been identified such as Candida glabrata, Candida dubliniensis, Candida guilliermondii, Candida krusei, Candida lusitaniae, Candida parapsilosis, Candida tropicalis and Candida kefyr.[1]

CHROMagar is a one such novel, differential culture medium that facilitates the isolation and identification of multiple candidal species on the basis of colony color.[2]

An attempt has been done in this study to isolate and culture multiple candidal species by using CHROMagar and to evaluate whether radiation dose is directly related to increase in candidal species in the oral cavity of oral squamous cell carcinoma (OSCC) patients undergoing radiotherapy.

Materials and Methods

Study sample size

The study included a total of fifty individuals diagnosed with OSCC who reported to the Department of Radiology, Kidwai Memorial Institute of Oncology, Bengaluru. The patients were examined, and data regarding age, gender, site of occurrence of OSCC, size of lymph node, distant metastasis and histopathological grading were obtained and documented. For the study purpose, the samples were divided into two groups. The first group included patients who were examined at time point 3rd week (n = 50) following radiotherapy and second group included same patients examined at time point 6th week (n = 50) following radiotherapy. The radiation doses at the 3rd and 6th weeks were also recorded and smear samples were obtained from lesional sites using sterile swabs [Figure 1]. The protocol for the present study was approved by the Institutional Ethics Committee.{Figure 1}

Sample collection

The study sample was collected from the patients after thorough clinical examination with their consent. Relevant case history was recorded and photographs were taken. The patient was asked to open the mouth wide open or as much as possible. With aseptic techniques, sterile swab was removed from its container and rolled over the lesional surface to collect the sample. The swab was again placed back immediately into the container to prevent any contamination and sent to the laboratory.

Laboratory methods

The collected swabs were inoculated onto CHROMagar plates and then incubated at 37°C for 48 h. The identification of candidal species was made by utilizing the colony color and morphological characteristic on the CHROM agar culture plates [Table 1]. The colony count was performed using a digital colony counter by placing the plates on the illuminating surface

of the digital colony counter. The magnifying glass on the digital colony counter was adjusted to have a good look of the colonies. The tip of the marker pen supplied along with the digital colony counter was touched over the colonies present on the plate, and the number of colony forming units (CFUs) per plate was counted. All the colonies on the plate were considered for total CFUs and individual Candida types were separated on the basis of colony color, and morphological characteristics and their CFU were also calculated.{Table 1}

Results

The present study constituted a total of fifty subjects. Swabs were collected from the lesional sites following radiotherapy at two intervals of time (time point at 3rd week and time point at 6th week).

Out of fifty patients, 31 (62%) were males and 19 (38%) were females, with a mean age of 54.74 ± 12.53 years (mean ± standard deviation). The patients were irradiated with a mean radiation dosage of 31.66 ± 0.35* and 61.54 ± 0.60* at 3rd week and 6th week, respectively (P = 0.00001). [Table 2] shows comparison of the mean of total CFUs (tCFUs) using paired t-test at time points 3rd week (58.36 ± 4.27)* and 6th week (100.26 ± 4.35)* and shows a significant correlation with P = 0.00001. The comparison of the mean CFU of C. albicans (green) [Figure 2] and [Graph 1], C. krusei (rose pink) [Figure 3] and [Graph 2], C. tropicalis (blue) [Figure 4] and [Graph 3] and C. glabrata (creamish white) [Figure 2] and [Graph 4] was done at time point 3rd week and 6th week using paired t-test. It showed a significant correlation with C. albicans (P = 0.038), C. tropicalis (P = 0.001), C. glabrata (P = 0.008) and insignificant correlation with C. krusei (P = 0.94) as shown in [Table 3].{Figure 2}{Figure 3}{Figure 4}[INLINE:1][INLINE:2][INLINE:3][INLINE:4]{Table 2}{Table 3}

Total radiation dosage at 3rd week was compared with the tCFUs at 3rd week, C. albicans colonies (green), C. krusei colonies (rose pink), C. tropicalis colonies (blue) and C. glabrata colonies (creamish white) using Karl Pearson's correlation coefficient method. Pearson's correlation shows a negative value (inverse relation) with respect to tCFUs at 3rd week (r = –0.0904) and C. glabrata colonies (r = –0.2043) and shows a positive value with respect to C. albicans colonies (r = 0.0074), C. krusei colonies (r = 0.0082) and C. tropicalis colonies (r = 0.0133). However, these findings were insignificant as P value was more than 0.05 as shown in [Table 4]. Similarly, total radiation dosage at 6th week was compared with the same variables as for 3rd week using Karl Pearson's correlation coefficient method which established a negative value (inverse relation) with all the variables showing insignificant correlation, with a P value of more than 0.05 as shown in [Table 5].{Table 4}{Table 5}

A significant (P < 0.05) direct correlation was established using Karl Pearson's correlation for time point 3rd week between tCFUs and C. albicans colonies (r = 0.4740), tCFUs and C. krusei colonies (r = 0.4085), tCFUs and C. tropicalis colonies (r = 0.4042) and tCFUs and C. glabrata colonies (r = 0.6377). However, for time point 6th week, a significant (P < 0.05) direct correlation was seen for all the variables as in for time point 3rd week, except for correlation between tCFUs and C. krusei colonies (r = 0.2100) which was insignificant (P > 0.05).

Discussion

Oral cancer is the sixth most common cancer worldwide. The development of OSCC is associated with habits such as chewing tobacco, betel quid, or areca nut along with alcohol producing a synergistic effect. The mutagenic effects of tobacco, betel quid, or areca nut and alcohol depend on dose, frequency, duration of use and are exaggerated by the concurrent use of two or more of these agents.[4] In India, oral cancer appears to be the most common cancer in males and the third most common cancer in females.[5] The present study reported a strong predilection for males (62%) than in females (38%), with a mean age of occurrence to be 54.9 in males and 54.4 in females.[6]

Radiations act on the nuclear DNA, leading to death or loss of its reproductive capacity. Radiotherapy has the advantage of organ preservation and is currently one of the primary modes of treatment used to treat OSCC.[7]

Radiotherapy to the head and neck region causes salivary gland tissue damage which leads to hyposalivation. This resultant decrease in salivary flow rate may contribute to decrease in potent antimicrobial levels, leading to the overgrowth of oral microbial species such as Candida. The studies conducted by Ramirez-amador et al.[8] and Nicolatou-Galitis et al.[9] have showed a definite positive correlation of radiotherapy with oral candidiasis. Subsequently, another study conducted by Redding et al.[10] showed 73% of candidal colonization in oral cavity of OSCC in patients receiving radiotherapy. A study conducted by Grötz et al.[11] also supported the work of Ramirez-amador et al.[8] and Redding et al.[10]

Oral candidiasis in OSCC patients is extensively explored using culture techniques, and numerous culture media are used for the isolation of candidal species such as cornmeal Tween 80 agar,[8] Sabouraud dextrose agar,[12] Pango-Levin medium,[13] and CHROMagar.[1] However, CHROMagar appears to be a promising selective culture medium for the growth of candidal species due to its efficiency to color and differentiate various types of Candida in culture plates. The present study utilized CHROMagar medium for culturing of candidal species at time point 3rd week and 6th week. It demonstrated numerous smooth green colonies, smooth creamish white colonies, rough blue colonies and fuzzy rose pink colonies representing C. albicans, C. glabrata, C. tropicalis and C. krusei, respectively.

Similar species were also identified by Nicolatou-Galitis et al.,[9] Belazi et al.,[1] Ramirez-amador et al.,[8] Azizi and Rezaei,[14] Mucke et al.,[15] Redding et al.,[16] Pasqualotto et al., [17] and Bakki et al.[18] The studies conducted by Ramirez-amador et al.,[8] Dambroso et al.,[19] and Suryawanshi et al.[12] showed a significant increase in candidal CFUs at various stages of radiotherapy. The present study also demonstrated a significant (P = 0.00001) increase in total candidal colonies at time point 3rd week and 6th week following radiotherapy.

The present study indicated a significant predominance of C. albicans at time point 3rd week (P = 0.0380), followed by C. glabrata (P = 0.0008). These findings were consistent with the studies conducted by Dambroso et al.[19] and Belazi et al.[1] This increase may be attributed to more prevalence of Candida albicans species in the oral cavities of both healthy and diseased[20] or it may be due to decreased immune response of the oral mucosa as a consequence of the secretory dysfunction resulting due to radiotherapy or it may simply be a resultant of radiotherapy-related opportunistic infection of Candida in which C.albicans has taken a leading role.[21]

The present study also showed very low populations of C. krusei and C. tropicalis at the time point 3rd week, which was consistent with studies as that of Redding et al.,[16] Belazi et al., [1] and Nicolatou-Galitis et al.,[9] all of which demonstrated a relatively low number of C. krusei and C. tropicalis in the oral cavity of OSCC patients undergoing radiotherapy. However, the present study showed a significant drastic increase in C. tropicalis population at time point 6th week.

Non-C. albicans Candida (NCAC) species have been described as emerging species which cause fungal infections in immunocompromised hosts.[22] Redding et al.[10] for the first time reported oral mucosal infection by C. glabrata in head and neck cancer patients undergoing radiotherapy. In a study, Dambroso et al.[19] demonstrated a higher frequency of NCAC species (C. glabrata, C. tropicalis, C. parapsilosis) in the oral cavity of head and neck cancer patients and stated that ionizing radiation not only enhances the frequency of Candida colonization but also enables colonization by species that are normally absent in healthy hosts. In the present study, the drastic increase in C. tropicalis and C. glabrata is in par with findings of Dambroso et al.[19] and such an increase may be reflected upon ionizing radiation.

Various studies indicate the relative increase in Candida colonization when the radiation dosage is increased.[19] The study conducted by Jham et al.[23] showed that the combination of radiotherapy and chemotherapy indicated a significant increase in oral Candida colonization. Another study conducted by Dahiya et al.[24] indicated an increase in candida colonization in OSCC patients with neutropenia receiving radiotherapy. Further, the study conducted by Jham et al.[25] demonstrated that the candidal colonization in the oral cavity of OSCC patients undergoing radiotherapy was independent of radiation dosage. The present study is in favor of the study conducted by Jham et al.[25] The above findings suggest that radiotherapy alone may not influence candidal growth in the oral cavity of OSCC patients undergoing radiotherapy. The candidal growth in the oral cavity may also be related to other factors such as chemotherapy, genetic background and the physiological state of the host,[26] several fungal survival factors,[27] influence of environmental condition in which the fungi are growing,[28] radiation tolerance of salivary gland,[29] and patient-to-patient variation of threshold levels.

Conclusion

The present study demonstrates an increase in candidal colonization in the oral cavity of OSCC patients undergoing radiotherapy from 3rd week to 6th week. However, the radiation dosage did not show any definitive significant correlation with candidal colonization in the oral cavity. Thus, candidal colonization in the oral cavity of OSCC patients undergoing radiotherapy is independent of radiation dosage and may be attributed to various confounding factors.[8],[26],[27],[28] The study also demonstrated a drastic increase in C. tropicalis and C. glabrata population in the 6th week following radiotherapy which favors the point that ionizing radiation influences the colonization of NCAC. However, further studies are required to validate these findings with relatively larger sample size.

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Conflicts of interest

There are no conflicts of interest.

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