

Oral Microflora among Oral Cancer Patients Undergoing Radiotherapy in Regional Cancer Center, Indira Gandhi Medical College, Shimla

Abstract

Objective: The objective of this study was to identify the microflora, especially Gram-positive, Gram-negative, and *Candida* species, in patients with oral squamous cell carcinoma during various stages from diagnosis through radiotherapy. **Materials and Methods:** A total of 17 cases with histological diagnosis of squamous cell carcinoma of the oral cavity were enrolled in the study. For each patient, the sample was collected thrice, i.e., at the time of diagnosis (Sample 1), 14th–15th day (Sample 2), and on the 29th–30th day of radiotherapy (Sample 3). The swab stick was rolled across the oral mucosa in the cases and was sent immediately to the Department of Microbiology, Indira Gandhi Medical College, Shimla, for processing. The swabs were inoculated on MacConkey agar, blood agar, and Sabouraud dextrose agar. After overnight incubation at 37°C, the organisms were identified by colony characteristics, catalase, coagulase test, Gram staining, and standard biochemical tests. **Results:** Out of 17, there was a loss to follow up in three patients, so after analyzing on 14 patients, we had 12 (85.7%) males and 2 (14.3%) females. The mean age of the population was 47.6% ± 12.2%. We had significantly higher proportion of Gram-positive microorganisms in Sample 1 as compared to Sample 3 and the same proportion of Gram-negative organisms in Sample 1 and Sample 3. *Candida* species was also proportionately higher in Sample 3 as compared to Sample 1. **Conclusion:** There is a shift of oral microflora from Gram-positive to *Candida* species from Sample 1 to Sample 3 and Gram-negative being same in Sample 1 and Sample 3.

Keywords: *Candida*, Oral cancer, radiotherapy and oral microflora

Introduction

Oral and oropharyngeal carcinomas are the sixth most common cancers in the world.^[1] In India, oral cancer is one of the most common cancers and constitutes a major public health problem. The incidence rates of cancers of the oral cavity in both males and females in all urban cancer registries of India are among the highest in the world.^[2] Oral cancers have a significant impact on the patient's quality of life because of the functional loss that results with the treatment modalities even with the highest care rendered nowadays.

Oral cancers have a multifaceted etiology. Although smoking, tobacco chewing, and alcohol consumption are widely associated with oral cancers,^[1] these traditional risk factors alone fail to explain the changing epidemiological pattern of increasing incidence of oral cancers in many parts of the world.^[3]

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Most of the oral cancers are very much advanced at presentation and present a formidable challenge with regard to management to the oral surgeon. With the continuing advances in the techniques and modalities of radiation therapy, a large percentage of head-and-neck tumors are treated by radiation alone or by a combination of radiation and surgery. As it is less disfiguring and disabling, radiation therapy is usually the more desirable modality of the treatment for head-and-neck cancer, provided of course that there is a choice of therapy.

Mucositis and salivary gland hypofunction continue to be inevitable outcomes of radiotherapy.^[4] It is thought that the incidence and severity of radiotherapy-associated mucositis is caused in part by changes in the oral bacterial microflora. Oral microorganisms, especially Gram-negative, are believed to be involved in the ulceration phase, where they probably intensify the inflammatory

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process and aggravate or promote the formation of ulcers.^[5] Further, ulceration acts as a portal of entry for microorganisms into the bloodstream leading to local and systemic infections.^[6,7]

Several studies have shown abnormal flora in head-and-neck radiotherapy patients before and after treatment.^[8,9] With this background, the present study was conducted to identify the microflora, especially Gram-positive, Gram-negative, and *Candida* species, in patients with oral squamous cell carcinoma during various stages from diagnosis through radiotherapy.

Materials and Methods

The present study was conducted in the Department of Radiation Oncology, Indira Gandhi Medical College, Shimla. The permission to conduct the study was taken from the head of the institute. After obtaining the informed consent from the patients, a total of 17 cases with proven histological diagnosis of squamous cell carcinoma of the oral cavity were enrolled in the study. For each patient, the sample was collected thrice, i.e., at the time of diagnosis (Sample 1), 14th–15th day (Sample 2), and on the 29th–30th day of radiotherapy (Sample 3).

The swab stick was rolled across the oral mucosa in the cases and was sent immediately to the Department of Microbiology, Indira Gandhi Medical College, Shimla, for processing. The swabs were inoculated on MacConkey agar, blood agar, and Sabouraud dextrose agar. After overnight incubation at 37°C, the organisms were identified by colony characteristics, catalase, coagulase test, Gram staining, and standard biochemical tests.

Inclusion criteria

1. Patients diagnosed with histologically proven oral squamous cell carcinoma scheduled for radiotherapy were included in the study.

Exclusion criteria

1. Patients on antibiotics
2. Patients on corticosteroid
3. Patients with any systemic disease that will bring about a change in the oral microflora were excluded from the study.

Statistical analysis

The data were analyzed by SPSS version 16 (SPSS Inc., Chicago, IL). The test used was Mc Nemar test. P value $\leq .05$ was considered statistically significant

Results

The study sample consisted of 17 participants. Out of 17, three were lost to follow up either in Sample 2 or Sample 3 and were thus excluded from the study. Hence, the analysis was done for 14 participants. Out of 14 participants, there were 2 females (14.3%) and

12 males (85.7%). The mean age of the population was $47.6\% \pm 12.2\%$ with a range of 29–65 years. We had a maximum number of participants in the age group of 56–65 years (35.7%). The most common site of squamous cell carcinoma was buccal mucosa in 8 (57.2%), followed by tongue in 4 (28.6%) and alveolus and lower lip in 1 (7.1%) each [Tables 1 and 2].

The distribution of organisms is shown in Table 3.

On comparing the Sample 1 and Sample 2, both had 21.4% of participants with Gram-positive microorganisms. Gram-negative organisms were found in 42.9% in Sample 1 and 35.7% in Sample 2 and *Candida* was found in 21.4% in Sample 1 and 14.2% in Sample 2, but the difference was not statistically significant [Table 4].

On comparing Sample 1 and Sample 3, Gram-positive organism was found in 21% in Sample 1 and 7.1% in Sample 3, and the difference was statistically significant ($P = 0.047$). Gram-negative organisms were found in equal proportions in both Sample 1 and Sample 3. *Candida* species was found in the higher percentage of participants in Sample 3 (35.7%) as compared to Sample 1 (21.4%), but the difference could not reach statistical significance [Table 4].

Table 1: Age- and gender-wise distribution

Age (years)	Number of participants (%)	Gender	n (%)
26-35	3 (21.4)	Male	12 (85.7)
36-45	3 (21.4)	Female	2 (14.3)
46-55	3 (21.4)		
56-65	5 (35.8)		

Table 2: Distribution of participants according to site involved

Site involved	n (%)
Buccal mucosa	8 (57.2)
Tongue	4 (28.6)
Alveolus	1 (7.1)
Lower lip	1 (7.1)

Table 3: Microorganisms present at various levels of radiotherapy

Organism	Sample 1 (%)	Sample 2 (%)	Sample 3 (%)
Alpha hemolytic streptococci	1 (7.1)	1 (7.1)	1 (7.1)
<i>Acinetobacter</i>	1 (7.1)	1 (7.1)	0 (0)
C species	2 (14.2)	2 (14.3)	4 (28.5)
Commensals	3 (21.4)	6 (42.9)	4 (28.6)
Cons	1 (7.1)	1 (7.1)	0 (0)
<i>Escherichia coli</i>	4 (28.5)	3 (21.4)	6 (42.8)
<i>Pseudomonas</i>	1 (7.1)	0 (0)	0 (0)
<i>Staphylococcus aureus</i>	1 (7.1)	0 (0)	0 (0)

Table 4: Comparison of microorganisms in various groups

Microorganism	Sample 1 (%)	Sample 2 (%)	P value	Sample 1 (%)	Sample 3 (%)	P value
Gram-positive	3 (21.4)	3 (21.4)	1.000	3 (21.4)	1 (7.1)	0.047*
Gram-negative	6 (42.9)	5 (35.7)	1.000	6 (42.9)	6 (42.9)	1.000
<i>Candida albicans</i>	3 (21.4)	2 (14.2)	1.000	3 (21.4)	5 (35.7)	0.275

*Statistically significant

Discussion

The present study tried to identify the change in oral microflora in patients with oral carcinomas at the time of diagnosis, 15 days after radiotherapy, and 30 days after radiotherapy. The results of the study show that at the time of diagnosis and 15 days after radiotherapy, Gram-positive microflora was found in 21.4% of participants which reduced to 7.1% after 30 days of radiotherapy. Furthermore, the *Candida* species increased from Sample 1 to Sample 3 though not significantly, so we can say that there was a shift in oral microflora from Gram-positive organisms to *Candida* species. These findings are in accordance with Almståhl *et al.*^[7] and Samaranayake *et al.*^[8] Several factors may contribute to the oral colonization by these microbial species, such as radiation-induced hyposalivation, smoking, alcohol abuse, and poor oral and corporal hygiene standards, which are commonly observed in most patients with head-and-neck cancer.^[9,10] In our study also, 28 (93.3%) participants had a positive history of tobacco use (60% in the form of smoking and 33% in the form of chewing) and 70% had a positive history of alcohol use.

Oral candidal carriage was reported by various authors in healthy as well as in individuals undergoing radiotherapy, the findings of which were consistent with the present study. The prevalence of yeast in the oropharynx of healthy volunteers was reported to be 35% by Cohen *et al.*^[11] and 38% by Ramirez-Amador *et al.* In the present study, *Candida* species was found in 21.4% in Sample 1 and 35.7% in Sample 3 which is lower than the findings of Ramirez-Amador *et al.*,^[12] who reported that *Candida* prevalence in patients increased from 43% in the first sample to 62% during radiotherapy and finally to 75% at the end of radiotherapy. The positivity could be due to the fact that the patients are often unable to maintain adequate nutritional status and oral hygiene during radiotherapy, in spite of receiving instructions and care.

Gaetti-Jardim *et al.*^[13] reported a higher proportion of Gram-negative organisms in Sample 3 as compared to Sample 1 which is in contrast to our study where we had the same proportion in Sample 1 and Sample 3, and in Sample 2, there was a slight reduction in Gram-negative microorganisms. Gram-negative bacteria such as *Enterobacter* and *Pseudomonas* are believed to be involved in the ulceration phase, where they probably intensify the inflammatory process and aggravate or promote the formation of ulcers by the release of proteases and endotoxins^[11] which interact with host cells to induce the

production and secretion of proinflammatory cytokines^[14,15] resulting in mucositis. These enteric microorganisms frequently act as reservoirs for antimicrobial resistance genes, which spread to other microbial populations.^[16] Further, ulceration acts as a portal of entry for microorganisms into the bloodstream leading to local and systemic infections.^[17]

The limitation of the present study is the small sample size of the study. Hence, more participants are required to increase the reliability of the results, and more detailed studies are necessary to understand the relationship between radiotherapy and alterations in the nature and magnitude of the oral microflora. Better characterization of changes in oral microflora would be obtained using molecular biological techniques. This would help our understanding of the potential role of oral microflora in the development and exacerbation of oral mucositis. Data from such work could be directed toward developing and testing selective antimicrobial therapies for the prevention and management of mucositis during cancer chemotherapy.

Conclusion

In this small-scaled patient study, we tried to investigate microflora at the time of diagnosis, in between radiotherapy, and at the end of radiotherapy. It was seen that there was a significant reduction in Gram-positive microorganisms at the end of radiotherapy as compared to the start of radiotherapy. There was no change in Gram-negative microorganisms at the beginning of radiotherapy and at the end of radiotherapy. Furthermore, there was an increase in proportion of *Candida* species though not statistically significant from the beginning of radiotherapy to the end of radiotherapy.

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Conflicts of interest

There are no conflicts of interest.

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