Evaluation of Alteration in Oral Microbial Flora Pre- and Postradiation Therapy in Patients with Head and Neck Cancer

Ayanambakkam Hemasundar Harini Priya1, Rajmohan2, Hari Priya Arun Kumar3, Shakthi Akash Raj4, Sai Archana5, Bharathwaj Venkatamarasu2

1Department of Oral Pathology, Chettinad Dental College and Research Institute, Chennai, Tamil Nadu, India, 2Department of Public Health Dentistry, SRM Dental College, Chennai, Tamil Nadu, India, 3Department of Oral Medicine and Radiology, SRM Dental College, Chennai, Tamil Nadu, India, 4Department of Prosthodontics, Meenakshi Ammal Dental College and Hospital, Chennai, Tamil Nadu, India, 5Department of Oral Medicine and Radiology, Chettinad Dental College and Research Institute, Chennai, Tamil Nadu, India.

Introduction: For decades now, head and neck cancer (HNC) remains to be one of the deadliest; per se the treatment for the same has been evolving for the past years. Radiation therapy (RT) has been one among the various treatment modalities adopted to treat HNC. The fact that RT can affect the quality of life in these patients cannot be denied. In patients with HNC, these can bring about a wide array of lesions in oral cavity and its associated structures as these areas are invariably affected due to exposure to radiation as such. These include alteration in salivary parameters, changes in microbial flora, and occurrence of radiation caries. Aim: In our study we aimed at assessing the alteration in oral microbial flora inclusive of Streptococcus mutans and Lactobacillus colony count before and after RT. Materials and Methods: Saliva and plaque samples were collected from patients with HNC, who were prescribed to undergo intensity-modulated radiation therapy (IMRT) amounting to a mean radiation dosage of 60 Gy units. The aforementioned samples were collected at three different intervals, before, immediately after, and 6 months after RT. The samples were cultured in selective media in three different dilutions (1:10, 1:100, and 1:1000). Following with colony counting is carried out using a standardized colony counter. Conclusion: The results showed a significant alteration in microbial flora pre- and post-RT. Individual parameters do not show a single stand in causing these changes. Moreover, these changes seem to have earned a multifactorial contribution.

Keywords: Head and neck cancer, Lactobacilli species, radiation therapy, saliva, Streptococcus mutans

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The principle aim of malignancy treatment was to treat the condition as early as possible to avoid recurrence and metastasis of primary lesion.\cite{6} The treatment ranges from surgery, chemotherapy to radiation therapy (RT).\cite{7} RT has evolved to an unimaginable extent; it dates back to 1900s.\cite{2} The principle advantage of RT compared to other treatment approaches is that it can provide long-term control of local or locoregional cancer without removal of large volume of tissue along with preservation of the adjacent normal tissues.\cite{3} Although RT has proved to be significantly associated with improved survival rate of patients with HNCs, the quality of life is still at stake. The long-term effects of RT pertaining to head and neck radiation oncology is inclusive of radiation caries (RC), xerostomia, radiation-induced mucositis, salivary gland dysfunction, craniofacial disturbances, nerve damage, osteoradionecrosis, and trismus.\cite{8} These changes can be due to direct or indirect effect of RT. RC remains as an important long-term effect of RT, which in turn can also be attributed to altered oral microbial flora.

**Materials and Methods**

The study was approved by the ethical board of SRM University. The samples were recruited from two private hospitals after obtaining due consent from the hospital authorities and the patients. Strict inclusion and exclusion criteria were followed so as to avoid errors from adjuvant factors.

**Inclusion criteria**
The inclusion criteria of the study included the following:

- Age group: 18 years of age or older
- Any gender
- Affected by HNC excluding lymphoma
- Four posterior teeth and four anterior teeth should be present
- No anticipated chemotherapy during the course
- Patients with HNC undergoing intensity modulated radiation therapy (IMRT) of dosage 60–66 Gy units only for 8 weeks.

**Exclusion criteria**
The exclusion criteria of the study included the following:

- Any systemic illness causing xerostomia
- Patients with removable or fixed appliances
- Receiving antibiotics during therapy or within 3 months before the study
- Edentulous patients

**Sample selection**
The samples were categorized into three groups

- Group I: Before RT
- Group II: After RT
- Group III: Six months after RT

**Sample collection**

Unstimulated saliva samples were collected under suitable aseptic conditions using routine protocol.

Unstimulated saliva was collected in sterile cups from patients with HNC.

pH of the saliva was estimated using pH meter at the chair side.

Saliva was then transferred to a graduated sterile saliva collecting tube using a 5-mL syringe and salivary flow rate was estimated.

The saliva sample was then centrifuged.
Supernatant was transferred into aliquot’s using micropipette and sent for microbial analysis.

Microorganisms are cultured using selective media to determine to isolate a selective organism as such and to determine its abundance in the sample being tested. The following two specific medias are used:

- Mitis salivarius-bacitracin (MSB) agar for *Streptococcus mutans*.
- Lactobacillus MRS agar (De Man, Rogosa and Sharpe agar) for *Lactobacilli* species

Duplicate samples were plated as 1 in 10 (1/10), 1 in 100 (1/100), 1 in 1000 (1/1000) dilutions, and spread on the surface of the medium. Surface plating method is usually adopted. The plates were then examined under a Digital Colony Counter. Paired *t* test was performed. The results were then tabulated.

**RESULTS**

Paired *t* test was performed to compare and estimate the difference between the groups.

Tables 1–3 and Graph 1 show evidence of statistically significant difference between Groups I and II, Groups I and III, and Groups II and III.

**DISCUSSION**

HNCs are often treated with RT, a precise technique that uses ionizing radiation. RT exerts therapeutic effect by scrupulously impairing the genetic material of susceptible malignant cells, either directly or via the release of free radicals by various mechanisms, thereby resulting in cell death.[9] RC can occur by two mechanisms, pertaining to etiology and pathogenesis: One is because of the direct effect of radiation so imparted on the tooth lying in the field of RT and the other is by indirect mechanism, attributed to the alteration in the salivary parameters (flow rate and pH), profound changes in the oral microbial flora (majorly *S. mutans*), and lack of maintenance of oral hygiene in radiotherapy patients.[10]

In this study, we aimed at analyzing the alteration in oral microbial flora pertaining to RC, which includes analysis of *S. mutans* and *Lactobacilli* colony count. Thus, our study showed a significant increase in *S. mutans* colony count before and after RT, before, and 6 months after RT. The elevation in colony count between Groups II and III, although statistically significant, was not as remarkable as former, which was in accordance with that of Brown *et al.*[11] The results so obtained were $\bar{x} \log_{10}$ count in the range of 2–3 before RT, which escalated to $\bar{x} \log_{10}$ count in the range of 3–4 after RT. On follow-up, the values further rose up to $\bar{x} \log_{10}$ count of more than 5 at the end of 3 months. Al-Nawas *et al.*,[12] found that the mean colony-forming units (CFUs) were approximately 630 ($\times1000$ cfu/mL) before RT, 920 ($\times1000$ cfu/mL) immediately after RT, and in the range of 1000 ($\times1000$ cfu/mL) 6 months after RT.

Meng *et al.*[13] have also showed similar results where the mean value before RT was log CFU 6.41 ± 0.81(607.89 ± 2.24 cfu/μL), immediately after RT was log CFU 6.76 ± 0.62 (862.64 ± 1.86 cfu/μL), and

| Variables | Mean | Standard deviation (±) | *P* Value |
|-----------|------|------------------------|-----------|
| Group I   | 548.00 | 37.05                  | 0.00 (*P* < 0.05) (significant) |
| Group II  | 971.50 | 223.36                 |

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| Group II  | 971.50 | 223.36                 | 0.00 (*P*<0.05) (significant) |
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6 months after RT was \( \log CFU 7.68 \pm 0.70 \) (1096.63 ± 2.18). The minor variation in CFUs among the studies might be attributed to the use of different agar medium for culturing the species.

Our study results were in discordance with those of the study conducted by Schuurhuis et al.\textsuperscript{[14]} wherein the study was conducted to compare the oral microbial flora on a long-term basis following RT using oral lavages. The results were in contrast to our study, in which the amount of \( S. \) mutans before RT was approximately 60\%-70\%, which reduced to 55\%-60\% after RT, and which further declined to 50\%-60\% at the end of 6 months after RT.

Similar trend was observed with estimation of \textit{Lactobacilli} colony count as evident in Tables 4-6 and Graph 2, wherein there was a significant increase in \textit{Lactobacillus} colony count before and after RT, before, and 6 months after RT. The rise in colony count between Groups II and III, although statistically significant, was not as remarkable as the other two.

The results of our study were in accordance with those of the study conducted by Brown et al.\textsuperscript{[11]} who found that the mean CFUs before RT were \( \bar{x} \log_{10} \) count in the range 1–2 before the start of RT, which escalated to \( \bar{x} \log_{10} \) count in the range 3–4 immediately after RT, raising to \( \bar{x} \log_{10} \) count in the range 4–5 3 months after RT. In a study conducted by Al-Nawas et al.\textsuperscript{[12]} the mean CFUs were approximately 575 (\( \times \)1000 cfu/mL) before RT, approximately 600 (\( \times \)1000 cfu/mL) immediately after RT, and approximately 920 (\( \times \)1000 cfu/mL) 6 months after RT. Vuotila et al.\textsuperscript{[15]} also conducted a similar study to analyze the salivary microbial alteration before and after RT. The results of the study showed a significant rise in \textit{Lactobacillus} levels after RT when compared to the values obtained before RT. The results of the study conducted by Schuurhuis et al.\textsuperscript{[14]} were found to be complex in nature, wherein the study was conducted to compare the oral microbial flora on a long-term basis following RT using oral lavages. The amount of \textit{Lactobacillus} before RT was approximately 70\%-80\%, which increased to 80\%-90\% after RT, which was in accordance with our study, but at the end of 6 months after RT the levels declined 50\%-60\%, which was in contrast to our results. The increase in \textit{Lactobacillus} level after RT is mainly due to the effect of radiation, which leads to release of reactive oxygen species, thereby creating an acidic environment, in

\begin{table}[h]
\centering
\begin{tabular}{lll}
\hline
Variables & Mean & Standard deviation (\( \pm \)) \\
\hline
Group I & 555.60 & 40.66 \\
Group II & 595.00 & 28.37 \\
\hline
\end{tabular}
\caption{Comparison of \textit{Lactobacillus} species (cfu/\( \mu L \)) between Groups I and II}
\end{table}

\begin{table}[h]
\centering
\begin{tabular}{lll}
\hline
Variables & Mean & Standard deviation (\( \pm \)) \\
\hline
Group I & 555.60 & 40.66 \\
Group III & 931.80 & 51.60 \\
\hline
\end{tabular}
\caption{Comparison of \textit{Lactobacillus} species (cfu/\( \mu L \)) between Groups I and III}
\end{table}

\begin{table}[h]
\centering
\begin{tabular}{lll}
\hline
Variables & Mean & Standard deviation (\( \pm \)) \\
\hline
Group II & 595.00 & 28.37 \\
Group III & 931.80 & 51.60 \\
\hline
\end{tabular}
\caption{Comparison of \textit{Lactobacillus} species (cfu/\( \mu L \)) between Groups II and III}
\end{table}
which the organisms multiply faster.[16] The significant fall in Lactobacillus count, 6 months after RT, might be due to two reasons: one is the samples used, where they have used oral lavages instead of saliva. Second, in this study the patients were subjected to an intensive oral care protocol and their oral hygiene improved substantially during follow-up, whereas in our study such protocol was not adopted.[14]

**CONCLUSION**

HNCs are more often treated by RT. Despite the fact that RT assumes a vital role in decimating tumor cells, its destructive symptoms cannot be ignored. RC is one such reaction, which happens on a long-run post-RT. Despite the fact that different investigations examined the immediate and aberrant impact of RT on tooth and its related structures, none were ended up being fruitful in defining a preventive measure to be embraced pre- and post-RT. In our study, we showed a significant alteration in oral microbial flora pertaining to dental caries, thereby suggesting use of antibacterial mouth rinses pre- and post-completion of RT on a long-term basis.

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**Conflicts of interest**

There are no conflicts of interest.

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