10.5005/jp-journals-10024-2359

ORIGINAL RESEARCH

Effect of Radiotherapy on Cariogenic Organism *Streptococcus sobrinus* in Saliva in Head and Neck Cancer: A Clinical Study

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ABSTRACT

Aim: Aim of the study was to assess salivary *Streptococcus sobrinus* in head and neck cancer using quantitative polymerase chain reaction (PCR).

Materials and methods: Unstimulated saliva samples were collected from head and neck cancer patient preradiotherapy. Unstimulated saliva samples were collected from oral and laryngeal cancer patients after 6 weeks of radiotherapy (dose 60 Gy). The subjects were explained not to consume solids or liquids or carry out any dental hygiene activity 1 hour prior to saliva collection. Accumulated unstimulated saliva was collected in cylindrical tube through funnel. The collected saliva was then transferred to Eppendorf tube containing Tris–ethylenediaminetetraacetic acid (EDTA) (TE) buffer and was transported to lab for real-time PCR analysis.

Results: *Streptococcus sobrinus* significantly increased postradiotherapy as compared with preradiotherapy in head and neck cancer patients.

Conclusion: Within the limitation of this study, we conclude that amount of *S. sobrinus* increases postradiotherapy in head and neck cancer patients.

Clinical significance: As radiation therapy has harmful effects on hard and soft tissues of oral cavity, dentists should provide motivation for oral health care to the patients.

Keywords: Dental caries, Oral cancer, Polymerase chain reaction.

How to cite this article: Kapoor S, Daveshwar SR, Sheth K, Daveshwar MR, Batra R, Agrawal V. Effect of Radiotherapy on Cariogenic Organism *Streptococcus sobrinus* in Saliva in Head and Neck Cancer: A Clinical Study. J Contemp Dent Pract 2018;19(8):929-932.

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Source of support: Nil

Conflict of interest: None

INTRODUCTION

The head and neck region of human body contains vital organs that are closely situated around each other. Malignant tumors of head and neck occur in the oral cavity, pharynx, paranasal sinuses, larynx, thyroid gland, parathyroid gland, salivary glands, bronchial tubes, and esophagus.[1] Irradiation is one of the modes of treatment used for cancers in these regions.[2] During treatment, irradiation affects the salivary glands and dentition, leading to many detrimental side effects, such as mucositis, xerostomia, and radiation caries. Radiotherapy can produce severe changes in oral microbiota.[3]

The major organisms leading to dental decay are *Streptococcus mutans*, *Lactobacilli*, and *Streptococcus sobrinus*.[4-6]

A few decades ago, most of the knowledge on the detection of the oral microbiome was based mainly on culture techniques.[7] More recently, PCR has been introduced. Different types of clinical samples of blood, sweat, semen, hair, and saliva are nowadays used for PCR analyses.[8] Saliva acts as a biomarker material for diagnostic tests in oral as well as systemic diseases. Investigations regarding bacterial and fungal profiles in saliva are popular and also have reliable results.[9]

In this study, quantitative real-time PCR was used for assessment of cariogenic *S. sobrinus* in saliva of head and neck cancer patients undergoing radiotherapy.

MATERIALS AND METHODS

Study population consisted of 30 head and neck cancer patients (15 oral cancer and 15 laryngeal cancer). All 30

Fig. 1: Eppendorf tube containing TE buffer

Fig. 2: Polymerase chain reaction machine

patients were screened preradiotherapy and postradiotherapy. Saliva was taken from the same individuals preradiotherapy and postradiotherapy.

- Group I: Preradiotherapy patients
- Group II: Postradiotherapy patients

For Saliva Sample Collection

Unstimulated saliva was collected from head and neck cancer patients preradiotherapy. Unstimulated saliva was collected from head and neck cancer patients immediately after 6 weeks of radiotherapy (dose 60 Gy). The subjects were explained not to consume solids or liquids or carry out any dental hygiene activity 1 hour prior to saliva collection.

Accumulated unstimulated saliva was collected in cylindrical tube through funnel. This collected saliva was then transferred to Eppendorf tube containing TE buffer (Fig. 1), which was transported to lab for real-time PCR analysis (Fig. 2).

The PCR Procedure

Real-time PCR analysis for detection of *S. sobrinus* was done at the Department of Microbiology, Maratha Mandal Nathajirao G. Helgekar Institute of Dental Science, Belagavi, Karnataka, India. The PCR procedure was as follows:

- The collected samples were transferred to the tube containing TE buffer.
- They were centrifuged at 5,000 rpm for 5 minutes.
- To it 500 mL fresh TE buffer was added with the help of sterile micropipette and it was centrifuged for 3 to 4 minutes and repeated 3 to 4 times.
- The supernatant was discarded and 50 mL lysis buffer I was added with the help of sterile micropipette and it was vortexed and kept for 5 minutes.

Then micropipette lysis buffer II for lysis of bacteria and 10 mL proteinase-K (100 µg/mL) for digestion of protein and removal of contamination from preparations of nucleic acid were added, and it was vortexed vigorously. Then it was kept in water bath for 2 hours and then in boiling water bath for 10 minutes.

Fig. 3: Specific PCR primers for *S. sobrinus*

The following set of PCR primers were used, which are specific to *S. sobrinus* (Fig. 3).

- Forward primer: GTFI-Forward 5′-GATAACTAC CTGACAGCTGACT-3′
- Reverse primer: GTFI-Reverse 5′-AAGCTGCCTT AAGGTAATCACT-3′

A mixture was prepared and aliquoted into each tube. The premix contained following components in a final volume of 20 µL/aliquot. (Qiagen Quantitect SYBR Green PCR master mix was used which contains 2.5 mM $MgCl_2$. In addition, the master mix contains Taq polymerase enzyme, dNTP mix, and SYBR Green dye.)

- Polymerase chain reaction master mix after thawing was gently vortexed and briefly centrifuged.
- A thin-walled PCR tube was placed on ice and the following components for each 50 µL reaction were added.
  - Quantitect SYBR green master mix: 10 µL

- *S. sobrinus* (Forward primer): 0.3 µL (10 pmol)
- *S. sobrinus* (Reverse primer): 0.3 µL (10 pmol)
- Template deoxyribonucleic acid (DNA): 3 µL (<1 µg/reaction)
- Water: Added to make final volume to 20 µlL

- The samples were gently vortexed and slowly the speed was reduced.
- The tubes were then placed in real-time thermal cycler (Eppendorf).

The PCR conditions were as follows:

Initial denaturation (95°C, 5 minutes)
Denaturation (95°C, 30 minutes)
Annealing (56°C, 1 minute) } 35 cycles
Extension (72°C, 1 minute)

RESULTS

The PCR Analysis

All the 30 samples showed presence of *S. sobrinus* in PCR readings. In the real-time PCR SYBR green method, the SYBR green dye binds with double-stranded DNAs which were specifically amplified by *S. sobrinus*-specific primers. The dye emits the fluorescence in the form of graph. The graph was plotted as the amount of fluorescence against the number of cycle. Serial dilutions of standard samples were run along with the test sample to plot the standard graph.

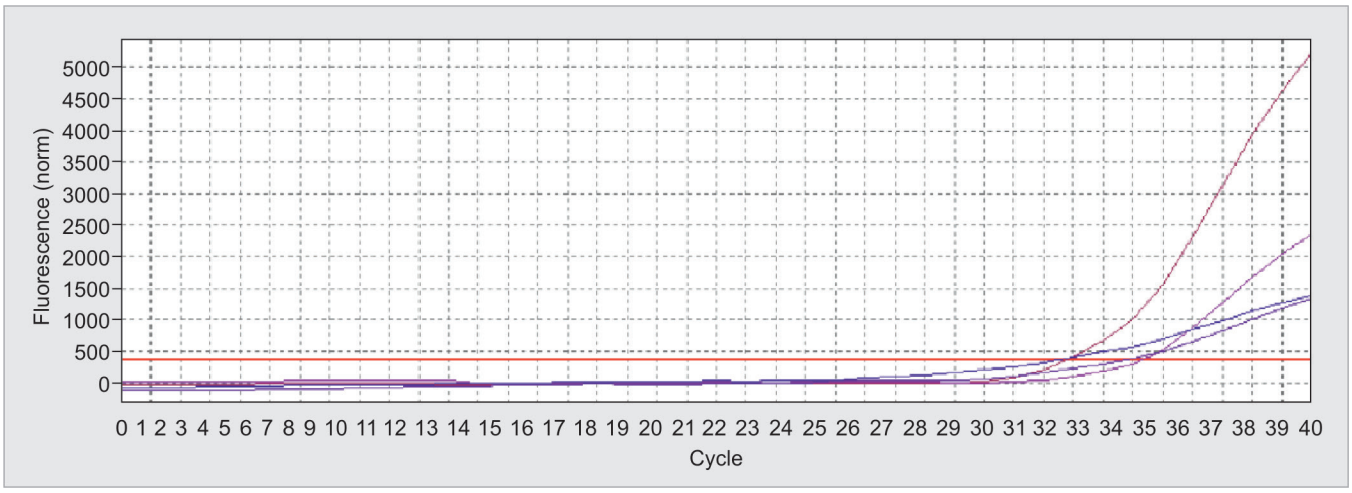
Realplex software graph results show increase in *S. sobrinus* from baseline postradiotherapy, as given in Graph 1. Statistical analysis was done using independent t-test to check *S. sobrinus* postradiotherapy (Table 1). The t-value of group II (2.151) was greater than group I (0.958). The mean difference of group II (3.81419) was also greater than group I (1.83729). The p-value of group I was 0.352. The PCR readings of group I were statistically insignificant as the t-value was near to 0 and $p > 0.05$. There was a statistically significant difference in PCR readings of group II as the p-value < 0.05. This depicted that *S. sobrinus* was significantly ($p < 0.05$) higher postradiotherapy as compared with preradiotherapy.

DISCUSSION

Radiation therapy in the head and neck region results in predictable reduced salivation. Irradiation damages the serous cells of salivary glands producing thick ropy saliva and markedly reduced salivation.

Xerostomia leads to dental and oral diseases. This occurs as there is an imbalance in normal oral cavity homeostasis, leading to increased dental caries and changes in taste, speech, eating habits; thus affecting life of an individual. Dental caries occurs due to complex interaction between acid-producing bacteria, fermentable sugars, and host factor.[10] Radiation caries is a destructive and rapidly progressing type of dental caries.

Kang et al[11] stated that presence of *S. sobrinus* in healthy individuals was lower as compared with individuals with head and neck tumors and the results were statistically significant. As not many studies are done on *S. sobrinus*, the present study was done on *S. sobrinus*.



Graph 1: Realplex software image indicating the amount of fluorescence against the number of cycles, and showing detection of *S. sobrinus* colony from baseline

Table 1: Statistical analysis of *S. sobrinus* in groups I and II

	t-value	*Degree of freedom*	*p-value*	*Mean difference*	*Standard error difference*	*95% confidence interval of the difference*	
						Lower	*Upper*
log_*S. sobrinus*_Pre	0.958	16	0.352	1.83729	1.91836	−2.22947	5.90404
log_*S. sobrinus*_Post	2.151	18	0.045	3.81419	1.77341	0.08840	7.53999

There is a high carcinogenicity of *S. sobrinus* compared with *S. mutans* as it is highly acid-forming organism. But *S. sobrinus* is detected only in a small number; *S. sobrinus* is commonly found along with *S. mutans*, where *S. mutans* are in majority compared with *S. sobrinus*. With the use of PCR, it can be concluded that there is a high prevalence of *S. sobrinus* than conventional culture methods. Although *S. mutans* are more in comparison to *S. sobrinus* in dental plaque,[12] the main cause for reduced growth and inadequate detection of *S. sobrinus* is due to its incapability to metabolize N-acetylglucosamine, which is an essential amino acid sugar found in oral cavity.[13]

The *S. sobrinus* proliferates when the high fermentable carbohydrates are consumed or there is an acidic oral environment.[14] In the present study due to radiotherapy, there was reduced salivary flow leading to acidic saliva, so there was increase in *S. sobrinus* postradiotherapy.

Plaque samples were not selected due to high variability of plaque coating and presence of cariogenic microbes on individual tooth surfaces.[15] Presence of saliva is in equilibrium to the oral cavity, thus making a good biomarker for microbial detection.[16]

Zhang et al[17] studied oral microbiota in nasopharyngeal carcinoma postradiotherapy patients. In this study, *Streptococcus* spp. was significantly higher postirradiation.

Hu et al[18] also found *Streptococcus* spp. significantly higher postradiotherapy in head and neck irradiated patients.

CONCLUSION

Thus, within the limitations of this study, it can be concluded that presence of *S. sobrinus* significantly increases in saliva postradiotherapy in head and neck cancer patients as compared with preradiotherapy patients.

CLINICAL SIGNIFICANCE

As the irradiation affects the hard tissue and increases the chances of dental caries, measures should be taken for oral health care. The measures that can be taken for oral health care are:

- Patient motivation.
- Changing the dietary habits. Frequency of sugar intake should be reduced and more of fibrous food should be taken.
- Fluoride mouthwash should be given to reduce the risk of dental caries.
- For xerostomia, salivary substitutes like cevimeline and pilocarpine can be given to the patients.

ACKNOWLEDGMENT

The authors provide sincere thanks to Dr Kishore Bhat (Director, Professor and Head of Department of Microbiology, NGH Institute of Dental Sciences and Research, Belagavi, Karnataka, India) for carrying out PCR work.

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