Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://www.elsevier.com/locate/aob

Exploring the variation of oral microbiota in supragingival plaque during and after head-and-neck radiotherapy using pyrosequencing

Li Gao, Yuejian Hu [1], *Yuxia Wang, Wenxin Jiang, Zhiyan He, Cailian Zhu, Rui Ma* [**], *Zhengwei Huang* [*]

Department of Endodontics, Ninth people's Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai Key Laboratory of Stomatology, Shanghai 200011, PR China

ARTICLE INFO

Article history:
Accepted 16 May 2015

Keywords:
Oral microbiota
Oral microecology
Supragingival plaque
Head-and-neck radiotherapy
Pyrosequencing

ABSTRACT

Objective: The aim of this article was to study the variation in oral microflora of the subgingival plaque during and after radiotherapy.
Design: During and after radiotherapy, microbial samples were collected at seven time points (early stage, medium stage, and later stage of radiotherapy, and 1 month, 3 months, 6 months, and 1 year after radiotherapy) in three subjects for a total of 21 samples. Polymerase chain reaction (PCR) amplification was carried out on the 16S rDNA hypervariable V1–V3 region, and then the PCR products were determined by high-throughput pyrosequencing.
Results: The rarefaction curve indicating the richness of the microflora demonstrated that the number of operational taxonomic units (OTUs) was in decline from the early stage of radiotherapy to the time point 1 month after radiotherapy and then trended upward. The Shannon diversity index declined during radiotherapy (ranging from 4.59 to 3.73), and generally rose after radiotherapy, with the lowest value of 3.5 (1 month after radiotherapy) and highest value of 4.75 (6 months after radiotherapy). A total of 120 genera were found; five genera (*Actinomyces*, *Veillonella*, *Prevotella*, *Streptococcus*, *Campylobacter*) were found in all subjects across all time points.
Conclusion: The richness and diversity of oral ecology decreased with increased radiation dose, and it was gradually restored with time.

1. Introduction

For head and neck cancer, radiotherapy is the main treatment method nowadays. However, in the treatment of cancer, ionizing radiation from radiotherapy also affects many normal organs, such as the salivary glands, mucosa, and teeth, inevitably causing certain oral complications such as mucositis,[1–3] hyposalivation,[4,5] osteoradionecrosis,[6,7] and radiation caries,[8–10] which adversely affect patients' quality of life.

Much research has been done on the effect of radiation therapy on head and neck cancer patients. To our knowledge, most studies have been focused on salivary velocity variation, radiation caries, and oral mucositis.[1–5,8–10] Some investigators

* Corresponding authors at: 639 Zhizaoju Road, Shanghai 200011, PR China. Tel.: +86 13764248949; fax: +86 21 63135412.
** Corresponding authors at: 639 Zhizaoju Road, Shanghai 200011, PR China. Tel.: +86 13024156520; fax: +86 21 63135412.
E-mail addresses: marui1723@sina.cn (R. Ma), huangzhengwei@shsmu.edu.cn (Z. Huang).
[1] The author contributed equally to this work.
http://dx.doi.org/10.1016/j.archoralbio.2015.05.006
0003–9969/

have found that these complications are associated with a change in oral microbiota. Sonalika et al.[11] compared the oral microbial change in squamous cell carcinoma patients at the time of diagnosis and during radiotherapy. They found candida to be increased in radiotherapy patients. Guobis[12] found that the level of hyposalivation and the duration of xerostomia have a significant impact on oral microflora. Actually, in the radiotherapy process, the major salivary glands are inevitably exposed to the radiation range.[13] Due to these glands' high sensitivity to radiation, the salivary secretion rate will continue to decline. Saliva plays an important role in the stability of oral physiological function.[14] Radiotherapy-caused hyposalivation would further undermine the balance of oral microflora system, which can also be called oral microecology. Almståhl[15] analyzed the microbial flora 6–8 months after completion of radiation therapy and found that *Candida albicans*, *Enterococci*, *Lactobacillus spp*, and *Streptococcus mutans* are distributed differently in different sites.

The above researchers obtained data mostly by the traditional culture method, and most of them focused on the microbial changes during radiotherapy. Now, culture-independent molecular methods such as pyrosequencing have more advantages than does the culture method.[16–18] Sakamoto et al. have revealed that the diversity of oral microbiota is more than previously demonstrated by traditional culture methods and that 40–55% of the bacterial taxa found in the oral cavity have not yet been cultivated and validly named.[18–20] A large number of sequence readings can be provided in a single run by pyrosequencing technology, resulting in a very large sampling depth and allowing detection of the most dominant microbial community members, even rare taxa.

In the investigation of microbial diversity, pyrosequencing has determined orders of magnitude higher than have previous technologies.[21,22] Therefore, more comprehensive microecological changes can be grasped by pyrosequencing than by traditional culture methods. To our knowledge, determining oral microecological changes during and after radiotherapy by pyrosequencing has seldom been reported. In previous studies,[23] we found that in radiation therapy, there was a negative correlation between species number and radiation dose. That is to say, the larger the radiation dose, the less diversity found in dental plaque. This suggests that microecological diversity exhibits dynamic changes during radiotherapy. In order to observe more microecological changes, this study was focused on the change of oral microecology during and after radiotherapy by pyrosequencing, and we compared the trends in microecological changes during and after radiotherapy. To our knowledge, there has been no such report.

2. Materials and methods

2.1. Enrollment and exclusion of subjects

This study was approved by the Ethics Committee of Shanghai Jiao Tong University. Appropriate written informed consent was obtained from all participating patients—those scheduled to receive head and neck radiotherapy in the Ninth People's Hospital affiliated with Shanghai Jiaotong University. None of the patients received antibiotics during and after radiotherapy or within 3 months before the study, nor did they have Sjogren syndrome or any disease characterized by xerostomia. Inclusion/exclusion criteria are listed in Table 1. An oral health examination was performed before radiation therapy. If necessary, restored the carious lesions, performed endodontic treatment and extracted doubtful teeth. The patients were given oral hygiene instruction, without any special fluoride remedy added.

2.2. Radiotherapy protocols and sampling time

The type of the radiotherapy is conventional radiotherapy (CRT). The protocol of radiation technique is as follows: Patients were placed in the supine position on a commercial head-neck thermoplastic mask attached to a carbon-fiber laminate base plate. CT images were acquired using a CT simulator. CT-based 3-dimensional treatment planning was used for the patients treated with CRT. The parotid and submandibular glands adjoined the target area so could not be

Table 1 – Admission criteria.

Admission criteria
Inclusion criteria
a. Presence of caries-free maxillary first molar in the oral cavity and absence of periodontal disease
b. Able to continue current diet and regimen of oral care for duration of the study
c. No anticipated chemotherapy during the course of the study
d. Life expectancy of at least 2 months
e. Eighteen years of age or older
f. Written informed consent
Exclusion criteria
a. Untreated cavitated carious lesions or oral abscesses
b. Periodontal pockets $\geq$4 mm
c. Clinically meaningful halitosis as determined by organoleptic assessment of an experienced clinician
d. Previous diagnosis of Sjogren's syndrome or any disease characterized by xerostomia
e. Receiving antibiotics during therapy or within 3 months before the study
f. Major salivary glands involved in the surgery region
g. Previous head and neck irradiation
h. Unable to maintain oral hygiene during the study