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Supragingival and subgingival microbiota from patients with poor oral hygiene submitted to radiotherapy for head and neck cancer treatment

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ABSTRACT

Objective: This case-control study aimed to evaluate the effects of conventional radiotherapy (RT) on the prevalence and populations of oral microorganisms in head and neck cancer patients who did not receive adequate preventive dental care. It was hypothesized that side effects of radiotherapy could be associated with radiation dose, microbiological aspects, and socioeconomic conditions of the patients.

Design: Twenty-eight dentate patients with head and neck cancer submitted to RT were included in the study. Radiation dose received varied from 4320 to 7020 cGy. Patients with the same demographic and health conditions, but no history of cancer or antineoplastic treatment were used as controls. Clinical examinations were carried out before RT, 15–22 days after starting RT, immediately after and 6 months after RT. Supra and subgingival biofilms were collected and cultivated onto selective and non-selective media. Isolates were identified by biochemical and physiological characteristics. Stimulated and unstimulated salivary flow rate and saliva buffer capacity were also determined.

Results: Mucositis, dermatitis, xerostomia, dysgeusia, dysphagia and candidiasis were common after starting RT and during the treatment period. Xerostomia was followed by a decrease in salivary pH and buffer capacity, which showed association with the increase of cariogenic cocci and yeast populations, which were also associated with deterioration of hygiene. *Candida* and family *Enterobacteriaceae* showed increased prevalence with RT, and were associated with the occurrence of mucositis and xerostomia.

Conclusions: Modifications in oral biofilms of irradiated patients showed association with xerostomia and hygiene conditions, which reinforces the necessity of improving patient compliance to oral health care programs.

1. Introduction

Radiotherapy (RT) is commonly used to treat head and neck cancer, significantly improving patient survival. However, RT is deeply associated with severe adverse reactions that may affect patient compliance, progress and intensification of the therapeutic regimen due to deterioration of the quality of life. The occurrence and severity of the side effects depend on dosage/frequency of RT, volume and anatomical aspects of irradiated tissues, age, oral and general health conditions of patients, as well as surgical procedures and associated chemotherapy (Minhas, Kashif, Altaf, & Nagi1, 2016).

Mucositis and xerostomia are the most significant and undesired effects of RT, affecting most irradiated patients, producing extreme discomfort. Xerostomia reflects the action of ionizing irradiation on the salivary glands and may exacerbate the effects of RT on highly proliferative epithelial cells of the oral mucosa, resulting in mucositis (Scarantino et al., 2006).

Reduction of the blood supply occurs in irradiated tissues during RT treatment. Thus, a similar reduction of the redox potential is expected, leading to wound repair delay and reduction of local immunity, which could particularly lead to proliferation of obligate anaerobes and microaerophilic bacteria in the supra or subgingival biofilms (Sonalika, Tayaar, Bhat, Patil, & Muddapur, 2012). Moreover, radiation-induced xerostomia seems to aggravate the prevalence of non-oral opportunistic pathogens in the oral biofilm of irradiated patients with adequate oral hygiene conditions and previous dental treatment (Almståhl, Wikström,

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& Fagerberg-Mohlin, 2008; Almståh, Wikström, & Fagerberg-Mohlin, 2015). However, in Brazil, most patients undergoing RT for cancer treatment present low socioeconomic conditions, inadequate oral hygiene standards (Avila-Campos, Ciesielski, & Sousa, 2011), and dental treatment is not a priority in many radiotherapy centers.

We hypothesized that the occurrence and severity of side effects of radiotherapy could be associated with radiation dose, microbiological aspects, and socioeconomic conditions of the patients. Hence, this study aimed to evaluate RT effects on oral microorganisms and opportunistic pathogens in subgingival and supragingival biofilms from head and neck cancer patients, attended or not by a preventive dental program, correlating the microbiological data with clinical parameters of irradiated patients.

2. Material and methods

2.1. Population and clinical examinations

This study was approved by the ethics committee of the School of Dentistry of Araçatuba (Process number 01559, UNESP, Araçatuba, SP, Brazil). Patients with head and neck primary malignant neoplasia, with no previous cancer treatment, at least eight natural teeth, not presenting any additional systemic disease and permitting intraoral and extra oral clinical examinations, and who signed the written informed consent were included in this study. All individuals were to start RT at the Regional Unit of Radiotherapy and Megavoltage (São José do Rio Preto, SP, Brazil) or were under clinical follow up at the Oral Oncology Centre (UNESP, Araçatuba, SP, Brazil). Patients with age less than 18 years, receiving bisphosphonates, drugs capable of affecting salivary flow, systemic medication with antimicrobial activity or steroids three months prior to the study, patients presenting history of previous cancer treatment or any pathosis affecting salivary gland function, exhibiting additional systemic diseases or with local factors capable to interfere with periodontal inflammation (excesses in the margins of restorations or crowns) and significant malocclusion were excluded (Almståh et al., 2015; Gaetti-Jardim et al., 2011).

Clinical evaluations involved dental and periodontal assessment with determination of number of teeth, fillings, crowns, bridges, probing depth, gingival recession, bleeding on probing (yes/no), plaque index (presence/absence) along gingival margin, and calculus deposits (Minhas et al., 2016; Gaetti-Jardim et al., 2011). The periodontal attachment loss was calculated by adding the probing depth and recession measurements, determined at six sites per tooth (mesio-buccal, buccal, disto-buccal, mesio-lingual, lingual and disto-lingual). After initial periodontal examinations, bitewing radiographs were taken.

Periodontally healthy subjects were those with absence of gingival inflammation, no bleeding on probing and no radiographic bone loss at all periodontal sites. Patients with gingivitis were those with signs of gingival inflammation involving at least 3 non-contiguous periodontal sites and absence of radiographic bone loss. Patients with chronic periodontitis evidenced attachment loss greater than 5 mm involving at least 3 non-contiguous periodontal sites and radiographic evidences of bone loss and moderate rate of progression of attachment loss.

The clinical trials were performed before RT, 15–22 days after starting RT, immediately after RT and 6 months after RT (Jham et al., 2007). Patients who eventually gave up treatment, or who could not be contacted, were replaced by other patients as the study progressed. The mean radiation dose received varied from 4320 to 7020 cGy (mean dose 6233 cGy ± 954.5 cGy) and the fractioning dose was 180 cGy. RT was performed using a linear accelerator and the major salivary glands were lying within the radiation field for all patients (Jham et al., 2007). The most frequent field irradiated was the right and left cervico-facial regions (78.6%).

Presence and severity of mucositis in patients was determined following the criteria provided by the National Cancer Institute. Summarily: grade 0 is the absence of clinical signs of mucositis; grade I, the mucosa is erythematous and painful; grade II, presence of pseudomembranes less than 1.5 cm in diameter and the patient feeds orally, consuming solid and semi-solid food; grade III, the presence of pseudomembranes greater than 1.5 cm in diameter and patient usually feeds liquid diet; and grade IV, evidencing the presence of ulcerations, making oral feeding impossible and requiring parenteral or enteral support.

The occurrence of xerostomia was determined 0by a specific questionnaire (Minhas et al., 2016) and clinical criteria, whereas hyposalivation was evaluated by sialometry (Scarantino et al., 2006). Measurements of salivary flow rates, buffer capacity and saliva pH were carried out as described by Almståhl et al. (2008). For sialometry of unstimulated whole saliva, all patients were instructed to refrain orofacial movements and to swallow the previous saliva present in their mouths. Then they allowed saliva to accumulate in the floor of mouth for 3 min and to spit the saliva into a preweighed sterile glass vial. This procedure was repeated more four times to provide data on 15-min collection. The weight of each collection was measured and adjusted to flow in ml/min for each patient. Values below 0.1 ml/min for resting saliva were indicative of hyposalivation.

To determinate the stimulated salivary flow, the stimulated secretion rate was measured using paraffin wax. The first saliva was swallowed and then all saliva secreted during 5 min was collected. This saliva was used to measure the pH and buffer capacity, using a pH/ion meter (Tecnopon mPA-210, Piracicaba, SP, Brazil). Buffer capacity of saliva was determined according to Ericsson's method (1959) (Ericsson, 1959).

Initially, a total of twenty-eight patients (20 males and 8 females) out of 183 patients, aged 18–68 years (mean age: 56.2 years), were included in this study. These patients harbored at least eight teeth (mean: 12.8 ± 4.5). Two subjects were periodontally healthy, ten had chronic periodontitis and sixteen presented gingivitis. Regarding the diagnosis of malignancy, twenty-six patients presented squamous cell carcinoma, one patient harbored acinar cell carcinoma and one presented basal cell carcinoma. Most patients were illiterate or had elementary formal education (85.7%), and were tobacco (92.9%) and/or alcohol (71.4%) consumers. The head and neck cancer treatment consisted of RT alone (42.9%) or combined with chemotherapy (10.7%), surgical resection (35.7%), or surgery and chemotherapy (10.7%). Patients were instructed to seek dental care before starting RT, but only 32.1% received dental treatment (dental extractions, periodontal treatment, and restorative procedures).

Each patient undergoing RT was matched according to age, sex, alcohol and tobacco consumption, periodontal and oral hygiene conditions with a control group with normal salivary flow rates and no history of head and neck cancer or cancer treatment. The control group patients were volunteers (18 males and 10 females) from the general public seen at the School of Dentistry of Araçatuba (UNESP, Araçatuba, SP, Brazil) aged 18–65 years (mean age: 52.4 years), harboring 14.2 ± 5.3 natural teeth; three were periodontally healthy; nine had periodontitis and sixteen presented gingivitis. All control group subjects presented an unstimulated salivary flow rate > 0.1 ml/min and a stimulated salivary flow rate > 1.0 ml/min and these values were considered normal (Almståhl et al., 2008).

After clinical examinations, patients received oral care instructions by dentists. In order to prevent radiation caries, patients were instructed to follow a protocol consisting of twice-daily use of 1100 ppm sodium fluoride toothpaste (Colgate-Palmolive Co., Sao Paulo, SP, Brazil) and daily aqueous fluoride (0.05% NaF). All irradiated patients who developed oral candidiasis were instructed to rinse oral mucosa with 5 ml of an oral suspension containing 100,000 U/ml aqueous nystatin three times a day (Almståhl et al., 2008) (Micostatin®, Bristol Myers Squibb, Sao Paulo, SP, Brazil).

2.2. Clinical samples

Subgingival and supragingival biofilm samples were collected before clinical examinations. Subgingival samples of the three most