Micromorphology of the Dental Pulp Is Highly Preserved in Cancer Patients Who Underwent Head and Neck Radiotherapy

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Abstract

Introduction: Teeth are often included in the radiation field during head and neck radiotherapy, and recent clinical evidence suggests that dental pulp is negatively affected by the direct effects of radiation, leading to impaired sensitivity of the dental pulp. Therefore, this study aimed to investigate the direct effects of radiation on the microvasculature, innervation, and extracellular matrix of the dental pulp of patients who have undergone head and neck radiotherapy. **Methods:** Twenty-three samples of dental pulp from patients who finished head and neck radiotherapy were analyzed. Samples were histologically processed and stained with hematoxylin-eosin for morphologic evaluation of the microvasculature, innervation, and extracellular matrix. Subsequently, immunohistochemical analysis of proteins related to vascularization (CD34 and smooth muscle actin), innervation (S-100, NCAM/CD56, and neurofilament), and extracellular matrix (vimentin) of the dental pulp was performed. **Results:** The morphologic study identified preservation of the microvasculature, nerve bundles, and components of the extracellular matrix in all studied samples. The immunohistochemical analysis confirmed the morphologic findings and showed a normal pattern of expression for the studied proteins in all samples. **Conclusions:** Direct effects of radiotherapy are not able to generate morphologic changes in the microvasculature, innervation, and extracellular matrix components of the dental pulp in head and neck cancer patients. *(J Endod 2014;40:1553–1559)*

Key Words

Cancer, dental pulp, radiation caries, radiotherapy, side effects

Head and neck squamous cell carcinoma (HNSCC) is one of the most common types of cancer in many parts of the world and has a significant impact on the quality of life of affected patients (1). Because of its local and regional pattern of growth, HNSCC is mainly treated by the combination of surgery, radiotherapy, and chemotherapy (1). In addition to the high rates of tumor control, head and neck radiotherapy (HNR) is associated with a series of severe oral toxicities. The main acute side effects of HNR are mucositis, hyposalivation, dysphagia, and dysgeusia, whereas the main chronic side effects of HNR include hyposalivation, trismus, osteoradionecrosis, and radiation-related caries (2).

During the medical planning of HNR, both tumor volume and normal anatomic structures of the maxillofacial region are included in the primary field of radiation (3). Thus, teeth often receive high doses of radiation during HNSCC treatment (4). Several *in vitro* studies analyzed the direct effects of radiation on the enamel and dentin and showed contradictory results regarding the negative impact of radiation on enamel dissolution, dentin collagen breakdown, and dentin bond strength (5–8). However, only a few previous studies attempted to investigate the direct effects of radiation on the dental pulp, most of which were based on animal experimental models and obsolete radiation techniques (5, 7, 9). Hence, there is great diversity of results when it comes to the ability of radiation to directly affect the dental pulp components (10–12) and to further impair pulp vascularization and innervation, leading to clinically detectable teeth sensitivity changes (13, 14).

Additionally, recent studies suggested that radiogenic destruction of the dental pulp would be an important event for the etiology of radiation-related caries (13). Remarkably, there is recent clinical evidence that HNR is able to reduce innervation and vascularization of the dental pulp by decreasing the levels of oxygen saturation, eventually decreasing pulpal sensitivity and responses to thermal and electrical stimuli (13, 14).

Considering the fact that responses of the dental pulp to thermal and electrical stimuli are directly related to the mechanisms of inflammation, microvascularization, and innervation of the dental pulp and given the scarce number of previous studies that explored the morphology of dental pulp in HNSCC patients, the current study tested the null hypothesis that the direct effects of radiotherapy would not be able to generate

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morphologic changes to the microvasculature, innervation, and extracellular matrix components of the dental pulp in HNSCC patients.

Materials and Methods

Patients

This study was approved by the Ethics Committee of the Piracicaba Dental School (protocol 116/2011), University of Campinas, Sao Paulo, Brazil, and the Ethics Committee of the Faculty of Medicine (protocol 191.171), University of Sao Paulo, Sao Paulo, Brazil. Twenty-three dental pulps derived from 23 teeth extracted because of advanced periodontal disease from 14 patients who had undergone HNR were included in this study. Irradiated teeth were extracted and stored according to the protocols previously described by Silva et al (15, 16). Nine (64.3%) patients underwent conventional bidimensional radiotherapy with parallel-opposed lateral fields, and 5 (35.7%) patients underwent conformal tridimensional radiotherapy. Radiotherapy plans from all patients were assessed for field of radiation and total tumor dose estimation. The electronic charts of the patients were retrieved for clinical and epidemiologic characterization, such as the patient's age, sex, tumor location, radiation field, total radiation dose delivered to the tumor volume, and time of tooth extraction after the conclusion of HNR, among others (Table 1).

Macroscopic and Radiographic Analyses

All specimens were reported according to their macroscopic characteristics and underwent photographic documentation. Each sample was codified and classified according to the anatomic group of origin. Subsequently, all specimens were radiographed in a standardized way. The teeth were placed individually on pieces of periapical radiographic film for adults (Kodak Ultra-speed; Eastman Kodak Company, Rochester, NY), with the crown facing the perforation of the film. An X-ray machine (Toshiba XR 6010 [127 V, 60 kV, 10 mA, and 60 Hz]) was used, with an exposure time of 0.3 seconds and focus-film distance of 15 cm. All radiographic films were processed in the same automatic processor.

Sample Preparation

All specimens were cleaned with periodontal curettes to remove residual soft tissue. Each tooth had been partially sectioned along the longitudinal axis with the aid of a diamond disk (Extec, Enfield, CT) and a low-speed saw (IsoMet 1000; Buehler Ltd, Lake Bluff, IL). Furthermore, with the aid of a dental chisel, the tooth was divided into 2 halves to remove the pulp entirely, with the aid of a surgical curette. After being removed from the teeth, the pulp samples were immediately fixed in 10% buffered formalin.

Microscopic Analysis

After fixation, the pulp samples were processed and embedded in paraffin. Three 5-μm-thick histologic sections were cut, stained with hematoxylin-eosin (H&E), and further scanned with the aid of the Aperio ScanScope CS system (Aperio Technologies Inc, Vista, CA). A descriptive microscopic analysis was performed by using all the tissue extension of pulp samples (from the coronal pulp to the apical end). The microscopic analysis focused on the presence and morphologic preservation of the following parameters: blood vessels, nerve bundles, and extracellular matrix components of the pulp (fibroblasts, fibrosis, calcification, necrosis, and inflammatory cells). The results generated by the morphologic study were analyzed by using descriptive statistics, absolute values, and percentages.

Immunohistochemical Analysis

Immunohistochemistry was performed on all samples following the methods of Vargas et al (17) and Carlos-Bregni et al (18). Briefly, the immunohistochemical reactions were conducted on 3-μm-thick histologic sections cut from the paraffin-embedded tissue blocks and mounted on silane-coated glass slides. Antigen retrieval was performed, and endogenous peroxidase activity was blocked using 10% hydrogen peroxide by incubation in 5 baths, each for 5 minutes. After being washed in phosphate-buffered saline (pH = 7.4), slides were incubated overnight with primary antibodies. All slides were subsequently exposed to avidin-biotin complex and horseradish peroxidase reagents (LSAB Kit; DakoCytomation, Glostrup, Denmark) and diaminobenzidine tetrahydrochloride (Sigma-Aldrich, St Louis, MO). Adequate positive control sections were used for each antibody, and the negative control was obtained by omitting the specific primary antibody. The antibodies, dilutions, and antigen retrieval methods used are described in Table 2.

The slides were analyzed by 2 oral pathologists who were previously calibrated. The immunoexpression of CD34 and smooth muscle actin was evaluated in the microvasculature; S-100, neurofilament, and NCAM/CD56 were evaluated in the innervation; and vimentin was evaluated in the extracellular matrix fibroblasts. A descriptive analysis was performed for these markers, and the results generated were analyzed by using descriptive statistics, absolute values, and percentages.

TABLE 1. Clinical Characteristics of Studied Patients

Patient	Sex	Age (years)	Smoker	Alcohol	Site	Stage	CH	RDT	Dose
1	M	50	Yes	Yes	Tongue	T2N2M0	Yes	LA 2D	60 Gy
2	M	59	Yes	No	Palate	T3N3M0	Yes	LA 2D	70 Gy
3	M	58	Yes	Yes	Tongue	T2N1M0	Yes	LA 2D	60 Gy
4	F	16	No	No	Nasopharynx	T3N2MX	Yes	LA 3D	70 Gy
5	F	54	Yes	Yes	Nasopharynx	T4N2MX	Yes	LA 2D	70 Gy
6	F	37	No	No	Nasopharynx	T4N3M0	Yes	LA 3D	70 Gy
7	M	49	Yes	Yes	Nasopharynx	TXN1M0	Yes	LA 3D	70 Gy
8	F	55	Yes	No	Larynx	T2N3MX	Yes	LA 2D	60 Gy
9	M	54	Yes	Yes	Larynx	T1N1MX	Yes	LA 2D	50 Gy
10	M	62	Yes	Yes	Oropharynx	T1N0M0	Yes	LA 2D	50 Gy
11	M	47	Yes	Yes	Larynx	T4N2MX	Yes	LA 3D	60 Gy
12	F	55	Yes	No	Oropharynx	T4N2M0	Yes	LA 3D	60 Gy
13	M	45	Yes	No	Oral cavity	T2N2M0	Yes	LA 2D	70 Gy
14	F	51	Yes	No	Nasopharynx	T4N2MX	Yes	LA 2D	50 Gy

CH, chemotherapy; F, female; LA, linear accelerator; M, male; RDT, radiotherapy.
Dose is the total radiation dose delivered to the tumor volume.