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Radiotherapy changes salivary properties and impacts quality of life of children with Hodgkin disease

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ABSTRACT

Objective: We aimed to perform a longitudinal investigation of the effects of radiotherapy on salivary flow rate, pH, buffering capacity, and protein composition of saliva and on the quality of life of children with Hodgkin disease.

Design: Ten children (6–16-year-old) with Hodgkin disease and 10 matched healthy children were investigated. Stimulated and non-stimulated saliva samples were collected at baseline, after 1080 and 2160 cGy of radiation, and 1, 2, and 3 months post-radiotherapy. The salivary flow rate was expressed as mL/min. Buffer capacity was determined by titration. Amylase activity, immunoglobulin A, mucin, and lactoferrin concentrations were determined by ELISA. Quality of life was assessed by Quality of Life – Head and Neck module 35 questionnaire.

Results: We found that radiotherapy caused hyposalivation at 1080 cGy and 1 month after radiotherapy and reduced buffering capacity at 2160 cGy. Mucin concentration and amylase activity in non-stimulated saliva increased 1 month after radiotherapy. Lactoferrin concentration increased during and after radiotherapy. Immunoglobulin A concentration increased at 1080 cGy, 1 and 2 months, for non-stimulated saliva and at 2160 cGy and 1 month for stimulated saliva. Children reported more pain after radiotherapy and more xerostomia during radiotherapy.

Conclusion: We concluded that the radiotherapy protocol affected the children's salivary properties and children's quality of life.

1. Introduction

Saliva is an important protector of the oral mucosa and teeth, due to its flow rate, buffering capacity, and protein composition. However, many factors can affect the production and composition of saliva. Hyposalivation, or reduced salivary flow rate (SFR), for example (Tschoppe, Wolgin, Pischon, & Kielbassa, 2010), is often associated with radiotherapy of the head and neck (Almstahl, Wikström, & Groenink, 2001), with a dose-dependent effect (Lee et al., 2006).

Compared with adults, children have greater number of cells in division, and these are very radiosensitive (Hall, 2006). Among children, Hodgkin disease (HD) is a rare type of cancer that affects the cervical lymph nodes, a condition that requires supraclavicular radiotherapy in case of partial response after chemotherapy. This treatment could affect the salivary glands in children and cause changes in salivary properties similar to those occurring in adults undergoing radiotherapy for the treatment of head and neck cancer (Imanimoghaddam, Rahrooh, Tafakhori, Zahedanaraki, & Homaeieshandiz, 2012). These changes can promote hyposalivation and xerostomia, both of which might affect the patient's quality of life. Moreover, radiotherapy can promote changes in protein patterns, such as an increase in the levels of lactoferrin (Almstahl et al., 2001), lysozyme, and immunoglobulins (Pajari, Poikonen, Larmas, & Lanning, 1989), probably due to tissue inflammation. Decreased concentrations and activity of α-amylase (Almstahl et al., 2001) and lower concentrations of mucin (Dijkema et al., 2012) have also been observed. These changes in saliva may make patients more susceptible to local infections.

To the best of our knowledge, there have been no studies investigating the salivary properties and quality of life of children

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with HD and undergoing radiotherapy. Therefore, this longitudinal study was undertaken to investigate the effects of radiotherapy on the flow rate, pH, buffering capacity, and protein composition of saliva, as well as on the quality of life of children with Hodgkin disease.

2. Materials and methods

This study was approved by the ethics committee of Piracicaba Dental School-University of Campinas (protocol: 15/2012) and by that of Boldrini Children's Center (protocol: 2:24-020412). To be included, the children should be undergoing radiotherapy based on the German Society of Pediatric Oncology and Hematology–Hodgkin's Disease (GPOH-HD95) protocol, with cervical involvement. Patients with neuromotor disabilities, cancer recurrence, or other characteristics that could affect salivary flow were excluded.

2.1. Patient samples

Children aged from 6 to 16 years and diagnosed with HD were recruited from Boldrini Children's Center, Campinas, São Paulo, Brazil between June 2012 and December 2013. Among 11 recruited children, 10 accepted to participate in the study. These individuals comprised the experimental group. For the control group, ten healthy children, also aged from 6 to 16 years, and non-medicated, were selected by the first author (L.M.L.) from public schools in Piracicaba, São Paulo, Brazil. For this group, the examination was performed at the schools. The age and gender of the individuals in the experimental and control groups were matched. Guardians who agreed to their children's participation signed an informed consent document.

Children in the experimental group were undergoing treatment according to the GPOH-HD95 protocol. After chemotherapy treatment, patients received daily radiation doses of 180 centigrays (cGy) up to 2160 cGy, by means of a Clinac 6EX linear accelerator and a 6 MV photon beam (Varian, Milpitas, CA, USA). All patients involved on the study received the same total radiation dose, however, they were submitted to different number of chemotherapy cycles. Of the 10 patients, 2 received two cycles, 3 received four cycles and 5 were sumitted to six cycles. These individuals underwent non-stimulated (NSS) and paraffin-chewing (Sigma Chemical Co., St. Louis, MO, USA) stimulated saliva tests. With regard to stimulated and non-stimulated saliva, a single saliva sample from each individual was collected at baseline, at doses of 1080 and 2160 cGy, and 1, 2, and 3 months after completion of the radiotherapy (Fig. 1). For the control group, saliva was collected only once.

2.2. Non-stimulated and stimulated salivary collection

Whole saliva was collected between 9:00 and 10:30 a.m., at least 1 h after food intake (Pontes, Polizello, & Spadaro, 2004). First, non-stimulated saliva samples was collected for 10 min and deposited in millimeter tubes. For stimulated saliva, samples collected in the first 30 s was discarded, and in the subsequent 5 min, all secreted saliva was collected. Both saliva samples were collected in an insulated icebox at 2–8 °C. For experimental group, saliva collection was performed at Boldrini Children Center, and for control group this procedure was carried out at school. The same person (L.M.L.) collected saliva samples for both groups. The saliva samples were then centrifuged at 12,000 rpm for 10 min and stored at −70 °C for biochemical analysis.

2.3. Salivary flow rates, pH, and buffer Capacity determination

The stimulated and non-stimulated SFR were measured (volume/collection time) and expressed as mL/min. Saliva (1 mL) was placed in Eppendorf tubes to determine its initial pH by means of a microelectrode Accumet® (Cole-Parmer International, Vernon Hills, IL, USA) coupled to a pre-calibrated Orion® pH meter (Thermo Scientific, Waltham, MA, USA). Buffer capacity was determined in 1.0 mL of saliva, and increments of 10 μL of 0.25 M HCl were added (Bouchoucha et al., 1997). At each increment, the tube was agitated and the pH determined, resulting in a pH slope. The area under the curve (AUC) was then calculated (pH × mmol HCl).

2.4. Salivary protein determination

The enzyme-linked immunosorbent assay (ELISA) was used in duplicate to quantify the salivary proteins. Salimetrics kits (Salimetrics, State College, PA, USA) were used to analyze the amylase and IgA. The USCN (USCN Life, Guangguguoji East Lake, Wuhan, China) and Abnova (Abnova Corporation, Neihu, Taipei, Taiwan) kits were used to quantify mucin 1 and lactoferrin, respectively. Following, the absorbance of each one of the 96-well-plate was evaluated by an EON spectrometer (BioTek Instruments, Winooski, VT, USA). Amylase activity values were expressed as unity of activity per milliliter (U/mL), and the concentration of IgA, mucin, and lactoferrin as nanograms per milliliter (ng/mL). These values were automatically calculated by spectrometer software based on the kit standard curve.

2.5. Assessment of children's quality-of-Life

The children's quality of life was assessed at each phase of the study, before saliva collection, by means of the Quality of Life – Head and Neck module (QLQ-H&N35) questionnaire, developed by the European Organization for Research and Treatment of Cancer (EORTC) (Aaronson et al., 1993). The Portuguese version of the questionnaire is composed by 35 questions about 12 domains: pain, swallowing, senses, speech, feeding, social contact, tooth, opening mouth, xerostomia, sticky saliva, couching and felt ill. The answers were scored from 1 to 4 (not at all, a little, quite a bit, very much) according to symptom intensity. Higher scores indicated worse symptoms. The questionnaire was applied to the children

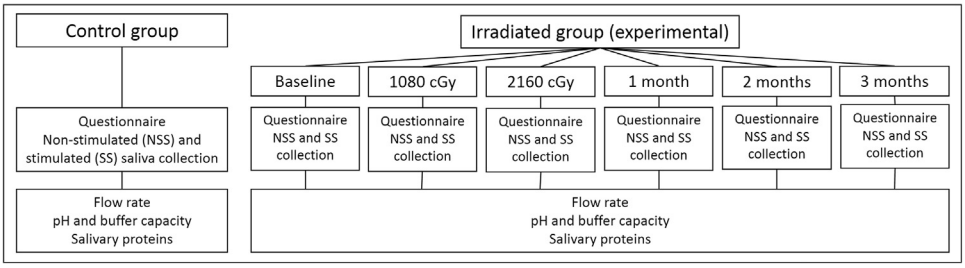

Fig. 1. Experimental design.