Original Article

Change in Surface Characteristics and Permeability of Human Enamel after Subjecting to Radiation Therapy

S Suri, N Raura, MS Thomas, PS Kumar[1], AJ Lewis[2]

Department of Conservative Dentistry and Endodontics and [2]Oral Pathology and Microbiology, Manipal College of Dental Sciences, Manipal Academy of Higher Education, Mangalore, Karnataka, [1]Department of Radiation Oncology, Medicover Hospitals, Nellore, Andhra Pradesh, India

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ABSTRACT

Background: Radiation-related caries is a complex destructive lesion leading to uncompromising damage of enamel and dentin in patients suffering from head and neck cancer managed with radiotherapy. **Aim:** The purpose of this study was to evaluate the changes in the permeability of enamel and to assess the morphological and chemical changes of teeth surface subjected to 6 MV photon beam irradiation. **Materials and Methods:** For this *in vitro* study, coronal portion of 20 premolars were sectioned mesiodistally into halves and then grouped into two. Samples in group 1 (control) were not subjected to cycles of irradiation and those in group 2 (experimental) were subjected to a cumulative uniform radiation dose of 70 Gray fractioned in 35 fractions with 6 MV photons. The silver nitrate penetration method was used to assess the change in permeability of enamel. The variations in surface topography and mineral content were assessed using scanning electron microscopy with energy dispersive X-ray analysis. Dye penetration scores of surface texture changes were compared between the two groups utilizing the Chi-square test. The change in the elemental levels between enamel surfaces of the two groups was compared using an independent *t*-test. **Results:** The application of 6 MV photon radiation did not change enamel permeability and surface topography. However, a noteworthy reduction in the carbon content ($P = 0.002$) was observed in teeth subjected to irradiation. **Conclusions:** Though radiation exposure did not alter the enamel permeability and surface topography, it had caused significant chemical compositional changes. Carbon content was significantly reduced in irradiated enamel samples.

Keywords: *Dental enamel, radiation effect, radiotherapy, scanning electron microscopy, tooth permeability*

Introduction

Head and neck cancers (HNC) comprise malignant tumors of the upper aero-digestive tract. Globally, in the year 2020, over 15,000,000 new cases of HNC were reported, and 500,000 people died.[1] Oral cavity is one of the most reported sites in HNCs, with tobacco being a critical etiological agent. Radiation therapy is the treatment modality commonly advocated for such cancers. Even though radiotherapy is curative, patients are generally susceptible to a wide range of complications like xerostomia, mucositis, trismus, soft tissue necrosis, dental caries, and osteoradionecrosis.[2]

"Radiation-related caries" is a late secondary effect of radiation therapy to the head and neck region.[3] Radiation-related caries is a damaging condition prompting serious dissolution of the inorganic components of the tooth in head and neck-irradiated patients and negatively affects their wellbeing and comfort.[4] The beginning and continuance of a carious lesion may be because of the immediate or roundabout

Address for correspondence: Dr. MS Thomas, Department of Conservative Dentistry and Endodontics, Manipal College of Dental Sciences, Manipal Academy of Higher Education, Mangalore, Karnataka - 575 001, India. E-mail: manuel.st@manipal.edu

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impact of radiation.[5] The indirect effects consist of trouble in performing appropriate oral cleanliness, alterations in the quantity and quality of saliva, and variations in the oral microflora. Radiotherapy can also cause direct effects on the tooth which may be associated with the pathogenesis of the disease, including enamel and dentin microhardness, alteration of the acid solubility, and crystalline structure of enamel.[6-8]

Enamel is the most mineralized tissue of the body, consisting primarily of carbonated hydroxyapatite (96%) with water (3%) and a very small amount of organic matrix (~1% wt.).[9] It forms a protective covering for the crown of the teeth. Any damage to the enamel integrity can affect the health of the teeth. Hence, the purpose of this study was to evaluate the effects of radiation on the topographical and chemical characteristics of the enamel of permanent teeth. The study also aims at assessing the changes in enamel permeability after radiation exposure.

Materials and Methods

This *in vitro* study was initiated after obtaining approval from the Institutional ethical committee and an approval number IEC/MCODS/19037 was assigned to it.

Sample selection, preparation, and storage

Twenty premolars extracted for the orthodontic purposes were selected for enamel permeability study. The sample size was determined using the resource equation and it also considered the possibility for sample loss during sectioning. Eight premolars were required for assessing enamel topography and surface composition postirradiation using scanning electron microscopy and energy dispersive X-ray analysis (SEM-EDX).

The crown of the chosen teeth was sectioned at the cementoenamel junction with the assistance of a low-speed diamond saw (Brasseler USA, Savannah, GA) at 300 rpm along with water cooling. The teeth were cleaned ultrasonically to eliminate all inorganic and natural materials adhered to the tooth surfaces. Teeth were then sectioned mesiodistally yielding two hemisections. Furthermore, to recognize enamel cracks, fractures, various imperfections, or caries, all the teeth were inspected under 8 X magnification (Carl Zeiss OPMI Pico, Germany). If any such defects were observed, those specific teeth sections were excluded from the study, and fresh teeth were included. The cleaned and polished specimens were then placed in distilled water until use.

Groups

The sections from each tooth were randomly divided into two groups:

Group 1: In this group, the samples were not subjected to cycles of radiation.

Group 2: In this group, the samples were subjected to cycles of radiation as described below.

The dental hemisections were put in culture plates loaded up with 10 ml of artificial saliva so the entirety of the samples got similar direct radiation for every unit zone. The artificial saliva was prepared according to Göhring *et al*.,[10] which consisted of hydrogen carbonate (22.1 mmol/L), potassium (16.1 mmol/L), sodium (14.5 mmol/L), hydrogen phosphate (2.6 mmol/L), boric acid (0.8 mmol/L), calcium (0.7 mmol/L), thiocyanate (0.2 mmol/L), and magnesium (0.2 mmol/L). The pH was maintained between 7.4 and 7.8. The synthetic saliva was replaced every 2 days.

Irradiation process

Only samples present in Group 2 were subjected to a cumulative uniform radiation dose of 70 Gray (Gy) fractioned in 35 fractions (2 Gy per fraction) with 6 MV photons. Treatment was delivered by Medical Linear Accelerator (M/S Elekta Medical Systems, Crawley, UK. Model: Compact) which was calibrated to deliver 1 cGy/MU for a field size of 10 × 10 cm with a dose rate of 350 MU/min following the protocol by the International Atomic Energy Agency dosimetry code of practice (TRS-398).[11]

Permeability assessment

Following the radiation process, 12 specimens were dried for 12 hours at room temperature. The samples were then coated with two layers of nail varnish on all the surfaces except for a window of approximately 3 × 3 mm on the middle third of the external enamel surface. This was to forestall the infiltration of silver nitrate (tracer) dye through the dentinal tubules. Samples were then submersed in 50% aqueous silver nitrate solution (Silver nitrate Pure, Merck Specialities, Private Limited, India) for 2 hours. The samples were photo-produced (Carestream Health India Pvt. Ltd., Mumbai, India) for 16 hours. They were then washed with water and hand instruments were utilized to eliminate the layer of nail varnish. Thin slices were obtained and photographed under a stereomicroscope of 100 X magnification (Reichert, Stereo Star Zoom-570). The scoring for the degree of dye penetration as per the study by Horning *et al*.[12] was as follows: Score 0- no dye penetration; Score 1—less than half the enamel thickness; Score 2—more than half of the enamel thickness; Score 3—the full extent of enamel without reaching the dentin; Score 4—tracer agent reaching dentin [Figure 1a–e]. The scores were carefully noted by two experts. If any disagreement, it was discussed, and a common consensus was reached.

Topographic assessment

After the radiation, samples from both the groups (eight segments in each group) were put away in artificial saliva for 24 hours. They were then gold-sputtered and analyzed for any surface changes utilizing SEM (LEO 440i, Carl Zeiss, Tokyo, Japan) under 1000x and 5000x amplification at selected three areas of the enamel surface. The consequences of SEM were analyzed and noted. Changes in the surface texture of teeth samples of groups one and two were recorded. Prismatic structure of enamel was scored as follows: (0) Regular rod-like structure, (1) slight change in rod structure, (2) moderate change in rod structure, and (3) severe change in rod structure. Interprismatic structural changes were scored as (0) unaltered, (1) slight alteration, (2) moderate alteration, (3) severe alteration.[13]

Chemical analysis

Chemical composition analysis of the samples was performed on the same specimens utilized for SEM. The dissemination of calcium, phosphorus, and other elements present in the enamel of human permanent teeth was assessed by EDX analysis. EDX energy-dispersive systems used spot measurement, element mapping, and EDX line scans. The differences in the results of the two groups were recorded and then studied.

Statistical analysis

All the data collected were tabulated and statistical analysis was performed using SPSS v20.0 software (IBM Corp, Somers, NY). Dye penetration scores and grades of surface texture changes were compared between the irradiated and the nonirradiated groups utilizing the Chi-square test. The change in the elemental levels between enamel surfaces subjected to radiation and those not irradiated were compared using an independent *t*-test. The level of significance was kept at 0.05.

Results

Enamel permeability using silver nitrate dye

The degree of penetration of silver nitrate dye varied between each sample irrespective of the exposure to radiation. There was no significant association in the dye penetration between the nonirradiated and the irradiated group indicating that the enamel permeability had no significant change after subjecting it to radiation therapy [Table 1].

SEM analysis of surface topographic changes of enamel

A SEM was utilized to get to the alteration in surface topography of enamel. The images show solid surface without individual enamel rods or prisms as in the case of aprismatic enamel [Figure 2]. No significant difference was observed in the surface topography between the nonirradiated and the irradiated enamel.

Table 1: Association between nonradiation group and radiation group based on dye penetration

Group	Dye penetration	Frequency	Chi-square value	P
Nonradiation group	0	4 (33.3%)	0.4762	0.976
	1	5 (41.7%)		
	2	1 (8.3%)		
	3	1 (8.3%)		
	4	1 (8.3%)		
Radiation group	0	3 (25%)		
	1	5 (41.7%)		
	2	1 (8.3%)		
	3	2 (16.7%)		
	4	1 (8.3%)		

Table 2: Descriptive statistics of elements (weight %) and their relation between nonradiation group and radiation group

Elements	Nonradiation group		Radiation group		*t*	*P*
	Mean	SD	Mean	SD		
Ca	30.175	5.909	33.628	8.252	1.178	0.251
P	15.765	2.016	16.915	2.229	1.326	0.198
C	14.393	2.275	11.675	1.313	3.585	0.002
O	34.293	6.707	32.480	9.165	0.563	0.586
F	0.498	0.271	0.507	0.109	0.108	0.915
Cl	0.755	0.142	0.831	0.186	1.133	0.270
Others	4.094	3.233	3.961	3.386	0.098	0.923

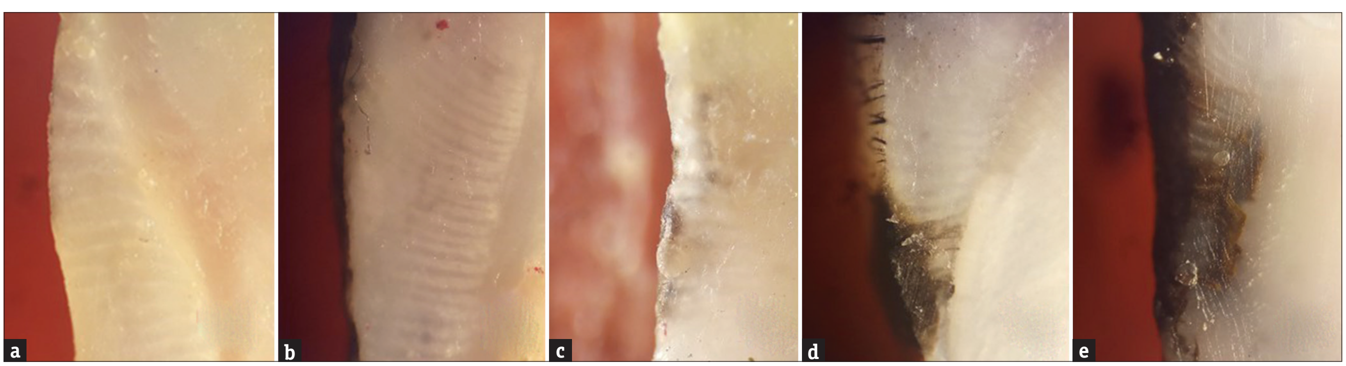

Figure 1: Scoring criteria for enamel permeability; (a) Score 0: no dye penetration (b) Score 1: penetration less than half the enamel thickness (c) Score 2: more than half the enamel thickness (d) Score 3: full extent of enamel without reaching dentin (e) Score 4: penetration into dentin

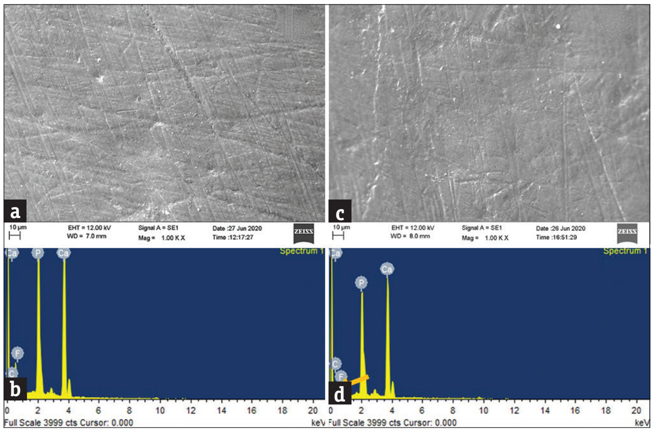

Figure 2: Representative scanning electron microscopic (SEM) image (a) of nonirradiated enamel surface (a) with EDX analysis (b) and corresponding SEM image (c) and EDX analysis (d) of irradiated enamel surfaces [*Note the orange arrow in panel d depicting the reduction in carbon content]

EDX analysis

The elemental analysis showed no substantial change in calcium and phosphorus concentration between the two groups. However, a noteworthy decrease in the carbon content in the group exposed to radiation therapy (P = 0.002) was observed [Table 2].

Discussion

Radiotherapy is one of the key systems for the treatment of HNC. An aggregate portion of 70 Gy radiation fractioned in 35 divisions (2 Gy for per fraction) was conveyed in the current investigation.[7] High-dose radiotherapy that destroys the malignant growth cells can prompt a few difficulties in the oral cavity including xerostomia, loss of taste, radiation caries, trismus, and breakdown of dentition.[2] While some complications associated with radiation have transient effects, the breakdown of dentition begins by the first year postradiotherapy and adversely impacts the well-being and comfort of the patient.[4] A few examinations have revealed that radiation doesn't cause direct impacts on the inorganic structure of human teeth, and the dental complications in patients going through radiation treatment are a result of the modifications in the natural organic framework of the enamel.[14]

In the current investigation, the enamel permeability, surface topography, and basic elemental examination were performed to assess the impacts of radiation on enamel. The utilization of silver nitrate to evaluate the permeability of tooth structure after bleaching therapy has been effectively demonstrated by Iwamoto *et al*.[15] Silver nitrate dye was used as a tracer in the study as it reacts with the reducing substance of the developer chemically and hence no dissolution takes place. Silver particles penetrate the porosity or the peripheral imperfections or defects on account of their small size by capillary activity, following which, ionic silver is reduced to metallic silver by a technique like the development of a photographic film.[16,17] The results of the dye penetration did not yield significant results between the nonirradiated and irradiated groups. Varied levels of dye penetration were observed in the samples after radiation. This could be attributed to the inherent cracks or defects present in the enamel causing the penetration of the dye.

The surface topography was studied with the help of SEM images of the two groups. Previous studies have shown that that radiation causes substantial morphological modifications in the interprismatic area.[6-8,18] It is conceivable that these modifications in the interprismatic region comprising of water, result from the aggregation of reactive oxygen species and free radicals which could respond with and impair the organic constituents.[12] In any case, as indicated by the consequences of this investigation, there was no huge change in the surface topography of enamel after radiation. This is contradictory to the results of the study by Kudkuli *et al*.,[18] where the enamel microstructure showed micro porosities and loss of smooth homogeneous surface after radiation exposure of 80 Gy. However, in the current study, both the groups showed solid surface without individual enamel rods or prisms. This aprismatic layer could be seen because the samples were not flattened and polished before subjecting them to the SEM analysis, unlike the previous studies.[19] Further studies are required to assess the role of aprismatic enamel in protecting the underlying dental hard tissues from the deleterious effects of radiation.

The elemental analysis was done utilizing EDX analysis to study the concentration of calcium and phosphorus in the enamel of permanent teeth. The calcium and phosphorus levels didn't show a noteworthy change. This is similar to the study by de Barros da Cunha *et al*.[20] which demonstrated that radiation did not interfere with the enamel Ca and P content. However, in the present study, there was a critical decrease in the carbon content in the group exposed to radiation therapy. This result is in exact contrast to the study by Bohn *et al*.,[21] where the chemical composition of dental caries related to ionizing radiation was altered with a decrease in Ca and P and an increase in C. The crucial difference among these studies is that the samples tested in this study were intact enamel exposed to ionizing radiation, rather than teeth with radiation-related caries as in the previous study.

It is believed that there is an electrostatic interaction between the apatite crystals and the organic matrix involving apatite phosphate groups and protein side-chain carboxylate groups. Exposure of teeth with high-dose radiotherapy >60 Gy, provides necessary excitation energy to bring about loss of acidic phosphate groups and decarboxylation leading to the formation of carbon dioxide and thus reduction in carbon content.[22] Postradiation, the apatite-bound calcium ions, and protein matrix side-chain carboxylation groups are eradicated partially by decarboxylation. The changes related to decarboxylation could affect the structure, mineral composition, and subsequently the mechanical properties of enamel.[23,24] The effects of decreased carbon content postradiation need to be studied in the future to recognize its role in acid resistance of enamel and the formation of radiation-related caries.

Apart from EDX, other methods like inductively coupled plasma optic emission spectroscopy (ICP-OES),[25] Attenuated total reflectance/Fourier transform infrared spectroscopy (ATR/FTIR),[26] FT-raman spectroscopy,[27] electron probe micro-analyzer,[28] have been used to analyze the chemical composition of enamel.[29] Additionally, the medium used to store the samples also varied from deionized water, saline to artificial saliva.[25] The storage medium used and the methodology employed to assess the elemental content would contribute to the variations in the structural and chemical composition of enamel postirradiation among the studies. Therefore as mentioned in the systematic review by Douchy *et al*.,[29] to better understand the consequences of therapeutic radiation on the chemical and structural properties of dental hard tissues, more studies with standardized protocols will be required.

The limitation of this study was the inability to mimic various oral conditions that can affect the integrity of the enamel surface of irradiated patients, like oral pH alterations as well as dietary changes. Additionally, the teeth surface was subjected to a cumulative uniform radiation dose of 70 Gy using a 6 MV photon beam. The effect of enamel on other irradiation conditions was not evaluated. Hence, more research efforts are necessary to have an in-depth understanding of the effect of radiation therapy on enamel.

Conclusion

According to the employed methodology in the current study, no alteration in the permeability of enamel was seen after subjecting the teeth' surface to radiation. The surface topography was also not altered despite irradiating the enamel. However, there was a significant alteration in the chemical composition of enamel after it was irradiated. The carbon content of enamel was significantly reduced in the irradiated samples.

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Ethical approval

The research project was approved by the Institutional ethical committee and an approval number 19037 was assigned to it.

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Conflicts of interest

There are no conflicts of interest.

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