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TITLE: EFFECT OF RADIOTHERAPY ON THE MECHANICAL AND CHEMICAL STRUCTURE OF PRIMARY TEETH ENAMEL: IN-VITRO STUDY

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ABSTRACT

Aim: This study aimed to assess the dental complications associated with radiotherapy in primary molars treated like oncological pediatric head and neck patients receiving irradiation treatment.

Methods: Five primary molars were selected and assessed for microhardness, Energy Dispersive X-ray Analysis (EDXA), and Environmental Scanning Electron Microscopy (ESEM). The molars were then embedded in acrylic blocks exposing the buccal surfaces. The molars were subjected to pediatric Hodgkin lymphoma radiotherapy regimen. The same tests were conducted to assess the mechanical and chemical structure after irradiation.

Results: Radiotherapy treatment led to a decrease in surface microhardness and a reduction in the levels of phosphorus and fluoride within the enamel. However, there were no significant alterations in calcium (Ca) content or the Ca/P ratio of the enamel. Additionally, notable superficial morphological changes in the enamel were observed following irradiation.

Conclusion: Radiotherapy negatively impacts the microhardness, chemical composition, and surface microstructure of the enamel in primary teeth.

KEYWORDS: Pediatric head and neck cancer, Radiotherapy, Vickers microhardness, EDXA, ESEM

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INTRODUCTION

The incidence of pediatric head and neck cancer accounts for approximately 12% of all childhood cancers (**Albright *et al.,* 2002**) a figure that has since risen to 17.3% (**Schwartz *et al.,* 2015**). This increasing trend outpaces the overall rise in pediatric malignancies, presenting significant challenges in both effectively treating the disease and minimizing long-term treatment-related complications (**Spiotto *et al.*, 2021**).

The most prevalent types of head and neck cancers affecting children include Hodgkin's lymphoma, non-Hodgkin's lymphoma, thyroid cancer, rhabdomyosarcoma, nasopharyngeal carcinoma (NPC), neuroblastoma, and salivary gland tumors (**Asakage, 2022**).

Treatment options for these cancers typically involve surgery, chemotherapy, radiation therapy, or a combination of these approaches (**Baskar *et al.*, 2012; Chow, 2020**).

Radiotherapy is a key modality in managing pediatric head and neck cancer, as it is effective in controlling tumors locally (**Spiotto *et al.*, 2021**). However, patients undergoing radiotherapy often face both immediate and long-term oral complications (**Bhandari *et al.*, 2020**).

One of the most common oral issues observed in children receiving radiation for head and neck cancer is radiation-induced dental caries. This condition results in alterations to the crystalline structure and mechanical properties of dental hard tissues, significantly diminishing their acid resistance and increasing the susceptibility to cavities. Moreover, radiation caries progresses rapidly and is challenging to manage (**Dobroś *et al.*, 2016; De Miranda *et al.*, 2021**).

The severity and frequency of radiation caries depend on a combination of direct and indirect factors (**Lieshout *et al.*, 2014**). Indirect factors include changes in diet, oral microbiota, and difficulty maintaining oral hygiene (**Vissink *et al.*, 2003; Silva *et al.*, 2009**). Direct factors, on the other hand, render irradiated enamel more vulnerable to cariogenic challenges. These include reductions in saliva production and alterations in its composition, as well as changes in the quality and microhardness of dental tissues, such as enamel and dentin. In some cases, the irradiated enamel may even develop cracks, leading to significant dental damage (**Silva *et al.,* 2009; Soares *et al.,* 2010**); Additionally, the impact of radiotherapy on teeth may vary depending on the mineral and organic composition of the enamel or dentin (**Marangoni-Lopes *et al.*, 2019).**

The radiotherapy direct effects on the primary dentition are still unknown because studies remitting this issue were only carried on the human secondary teeth and in bovine teeth and limited studies have been addressing the primary teeth. Therefore, the aim of this experiment was to conduct an in vitro evaluation of the irradiation effects on the mechanical, chemical and the superficial microstructure properties of the primary teeth enamel.

Research Hypothesis

Radiotherapy has no differential impact on the mechanical, chemical, or microstructural morphology preservation of irradiated enamel in primary molars.

MATERIAL AND METHODS

Ethics considerations

This study adhered to ethical guidelines and received approval from the Research Ethics Committee (REC) at the Faculty of Dentistry, Cairo University, with approval number 21-6-22. Additionally, the National Cancer Institute's REC at Cairo University approved the study under number 2207-407-044.

Sample Size Calculation

The sample size was determined based on data from a prior study **(Lopes *et al.*, 2018)**. The study reported a mean difference of 0.013 between experimental and control groups and standard deviation of 0.006 within each group. To detect a mean difference of 0.013 between experimental and control groups with a statistical power of 0.8 and a significance level (α) of 0.05, a minimum of five subjects per group was required. The sample size calculation was validated by the Medical Biostatistics Unit, Faculty of Dentistry, Cairo University.

Sample collection:

Five primary human molars meeting inclusion and exclusion criteria were selected from the Pediatric Dentistry and Dental Public Health Department at the Faculty of Dentistry, Cairo University. Extracted teeth were stored in distilled water until use.

Inclusion Criteria:

- Primary molars
- Intact buccal tooth surfaces

Exclusion Criteria:

- Carious buccal pits
- Existing buccal restorations
- Buccal erosion or abrasion
- Surface defects or cracks
- Hypocalcified or hypoplastic teeth
- Severely damaged teeth

The teeth were cleaned, scaled ultrasonically to remove plaque and calculus and stored in clean containers with distilled water at room temperature, following OSHA guidelines **(Nawrocka and Łukomska-Szymańska, 2019)**. The buccal surface of each tooth served as the standardized test area. Teeth were mounted on acrylic blocks (1 cm height) with exposed buccal surfaces and stored in jars containing distilled water.

Groups:

Group 1 (Control): Specimens remained untreated. Baseline mechanical, chemical, and surface morphology assessments were conducted.

Group 2 (Irradiated): Control group specimens underwent radiotherapy, followed by reassessment and comparison with baseline data.

Mechanical assessment:

Microhardness was measured using a Vickers hardness tester (Wilson TUKON 1102, BUEHLER, Germany). A 100 g load (HV 0.1) was applied smoothly for 10 seconds without impact. After load removal, diagonal impressions were measured to the nearest 0.1 µm and averaged. Hardness (HV) was calculated as:

$$HV = 1854.4L/d^2$$

Where L is the load in gf, and d is the mean diagonal length in µm.

Chemical and morphological assessment

Enamel samples were analyzed using high-resolution environmental scanning electron microscopy (ESEM) and energy-dispersive X-ray analysis (EDXA) (ESEM Quanta FEG 250, FEI Company, Netherlands). Calcium (Ca), phosphorus (P), and fluoride (F) atomic percentages were measured, and enamel surface photomicrographs were obtained at 2000x magnification.

Irradiation procedure:

The irradiation protocol simulated pediatric radiotherapy for head and neck cancer. Specimens received a daily fraction of 180 cGy for five days per week, totaling 3060 cGy over 17 fractions in 3.2 weeks. The protocol was based on the German Society of Pediatric Oncology and Hematology-Hodgkin's Disease (GPOH-HD95) guidelines **(Marangoni-Lopes *et al.*, 2019, 2021)**. Radiotherapy was delivered using a medical linear accelerator (Electa Versa HD Linac, Serial No. 156733, Stockholm, Sweden) under supervision by a radiation oncologist at the National Cancer Institute, Cairo University.

Statistical analysis:

Statistical analyses were conducted using SPSS 16® (Statistical Package for Scientific Studies), GraphPad Prism, and Microsoft Excel. Data exploration was carried out using the Shapiro-Wilk and Kolmogorov-Smirnov tests to assess normality, confirming that the data followed a normal distribution. Consequently, comparisons between the control and irradiation groups were made using the paired t-test. A significance threshold was established at $p \leq 0.05$.

RESULTS

Microhardness assessment:

The minimum, maximum, mean, and standard deviation of microhardness for all groups are summarized in **Table 1**. A paired t-test was used for group comparisons, revealing a significant difference (P = 0.0001). The control group (285.39 ± 19.21) demonstrated significantly higher microhardness than the irradiation group (45.58 ± 10.77), with a mean difference of 239.81 ± 9.85.

EDXA evaluation:

A paired t-test was used to compare the groups, revealing significant differences in fluoride (P = 0.003) and phosphorus (P = 0.005), while no significant differences were observed in calcium (P = 0.36) or the calcium/phosphorus ratio (P = 0.37), this was illustrated in **Table 2**.

ESEM investigation:

A representative ESEM image (×2,000) of the enamel surface is shown in **(Fig 1)**. The ESEM images reveal that sound enamel (a) displays a well-organized structure with a smooth, flat surface and minimal striations. However, following irradiation therapy, the enamel's structure was significantly disrupted, exhibiting increased porosity and the presence of fissures, as observed in (b).

TABLE (1) Descriptive results of Vickers hardness in all groups:

	Control	**irradiation**	Independent t test						
			Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference		t	df	P value
					Lower	Upper			
N	5	5	239.81	9.85	217.09	262.52	24.35	8	0.0001*
Minimum	267.33	34.37							
Maximum	315.93	58.70							
Mean	285.39	45.58							
Std. Deviation	19.21	10.77							

Significant difference as P<0.05.

TABLE (2) Descriptive results of mineral content of enamel in all groups:

		Minimum	Maximum	Mean	Std. Deviation	Paired Differences					t	df	P value
						Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
									Lower	Upper			
F	Control	0.26	0.38	0.31	0.05	0.17	0.06	0.03	0.10	0.25	6.31	4	0.003*
	Irradiation	0.11	0.18	0.14	0.03								
P	Control	17.53	18.84	17.99	0.50	1.72	0.68	0.31	0.87	2.57	5.63	4	0.005*
	Irradiation	15.29	16.70	16.27	0.56								
Ca	Control	50.23	55.55	52.97	2.36	2.28	4.97	2.22	-3.89	8.44	1.02	4	0.364
	Irradiation	45.31	56.01	50.70	3.89								
Ca/P	Control	2.75	3.12	2.95	0.16	-0.2	0.39	0.17	-0.65	0.31	-1.01	4	0.371
	Irradiation	2.75	3.66	3.15	0.34								

Significant difference as $P \leq 0.05$.

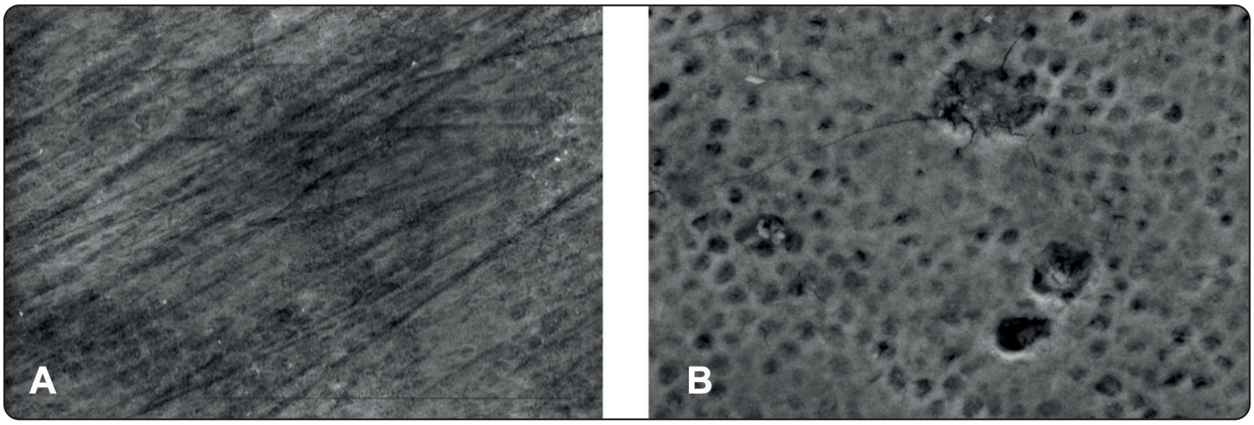

Fig. (1) Representative ESEM images of enamel surface appearance from different groups (×2000): (A) The control group, (B) The irradiation group.

DISCUSSION

Childhood cancer remains one of the most lethal diseases, affecting approximately 400,000 children and adolescents aged 0 to 19 each year, with many lacking adequate healthcare access **(Pergert *et al.*, 2020; Pathania, 2024)**.

Radiotherapy is often the primary treatment for early-stage cancer, serving as an alternative to surgical excision **(Saloura *et al.*, 2013; Barton *et al.*, 2014)**. Nearly half of all cancer patients receive radiotherapy during their treatment journey **(Baskar *et al.*, 2012)**.

While radiotherapy has advanced and achieved significant success, children treated for head and neck cancer often experience side effects, including severe and irreversible changes to the craniofacial region and oral cavity, such as radiation-induced dental caries **(Rio *et al.*, 2023)**.

This study focused on assessing the effects of radiotherapy on the mechanical, chemical, and microstructural properties of primary teeth.

Although this was an *in vitro* study, we simulated a radiotherapy protocol similar to that used in treating children with Hodgkin's lymphoma.

Considering the variation in enamel thickness between the buccal and lingual surfaces of teeth **(Gaboutchian *et al.*, 2023)**, the buccal surface was used in this study as a standardized test area to ensure uniformity in the chemical and physical properties of the enamel.

Microhardness is a critical property that reflects enamel's resistance to deformation and indentation **(Wu *et al.*, 2020)**. A strong correlation exists between enamel microhardness and mineral content **(Akkus., 2017)**. Therefore, assessing enamel microhardness and using SEM are essential for studying enamel demineralization and remineralization in caries research.

The Vickers microhardness test, commonly employed for evaluating dental materials, is efficient, requires minimal sample area, and provides reliable results **(Patil *et al.*, 2023)**.

Gold is known to block gamma radiation effectively **(Singh and Badiger, 2016)**. In this study, ESEM was utilized, allowing the same samples to be analyzed before and after irradiation **(Samaha and Gomaa, 2020)**. Furthermore, combined with EDXA, ESEM enabled a quantitative analysis of enamel composition and structural assessment **(Sathe *et al.*, 2014)**.

The null hypothesis of this study was rejected, as linear accelerator radiation significantly altered the enamel's morphology, chemical composition, and microhardness.

The baseline enamel microhardness values in this study (278.53±19.08 kg/mm^2) fell within the normal range reported by **(Ozgul *et al.*, 2015)**. However, irradiation caused a significant reduction in microhardness, aligning with previous findings **(Marangoni-Lopes *et al.*, 2019; Kudkuli *et al.*, 2020; Siripamitdul *et al.*, 2023)**.

This decrease is likely due to radiation-induced oxidation reactions that degrade water molecules in enamel, leading to reactive oxygen species that degrade collagen and non-collagen proteins, weakening the enamel **(Gonçalves *et al.*, 2014; Siripamitdul *et al.*, 2023)**.

Contradictory findings by **(Kielbassa *et al.*, 1999; Kielbassa *et al.*, 2000)** who reported that irradiation did not influence the micro hardness of enamel. This result was justified by **(Chambers *et al.*, 2007; Abdalla *et al.*, 2017)**, that in the studies by Kielbassa et al. the specimens were stored in saline, which stabilizes hydroxyapatite crystal surfaces and reduces enamel dissolution during irradiation. Conversely, under dry conditions similar to those experienced by patients with xerostomia, the absence of saliva increases enamel vulnerability.

Other studies reported increased enamel microhardness post-irradiation **(De Siqueira Mellara *et al.*, 2014; Gonçalves *et al.*, 2014; Reed *et al.*, 2015; Hindi *et al.*, 2016)**.

Regarding phosphorus content, EDXA showed a significant reduction after irradiation, consistent with **(Marangoni-Lopes *et al.*, 2019; Dos Santos *et al.*, 2024)**. This was explained as phosphorus, located on the enamel surface, is more susceptible to gamma radiation-induced damage as reported by **(Oliveira and Mansur, 2007; De Carvalho Filho *et al.*, 2011)**. However, other studies **(De Sá Ferreira *et al.*, 2016; Marangoni-Lopes *et al.*, 2021; Suri *et al.*, 2022; Siripamitdul *et al.*, 2023)** found no significant changes in phosphorus content.

Calcium and Ca/P ratio analyses revealed no significant differences between groups, consistent with findings by **(De Sá Ferreira *et al.*, 2016; De Barros da Cunha *et al.*, 2017; Kudkuli *et al.*, 2019; Suri *et al.*, 2022; Siripamitdul *et al.*, 2023)**.

However, others **(Duruk *et al.*, 2020; Dos Santos *et al.*, 2024)**, reported decreases in Ca and Ca/P, while **(Lu *et al.*, 2019; Morad *et al.*, 2024)** observed increases in Ca/P ratios post-radiotherapy.

Fluoride content decreased after radiotherapy, as reported by **(Morad *et al.*, 2024).** Regarding the results of the enamel surface analysis via ESEM , it was revealed micro-morphological alterations, including crack lines, micro-porosities, and irregular prism structures, in agreement with **(Marangoni-Lopes., 2019)**. However, other studies **(Soares *et al.*, 2011; Lopes *et al.*, 2018)** observed no such changes.

CONCLUSIONS

Exposure to radiotherapy induced significant changes in enamel's chemical and physical properties, including alterations in micro-morphological structure, mineral content, and microhardness.

RECOMMENDATIONS

Preventive strategies and oral hygiene measures are crucial before and after radiotherapy to mitigate enamel demineralization, enhance remineralization, and reduce radiation-induced dental caries in children.

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