Original Article

The Effect Of Fluoride Varnish Application On Microhardness And Structural Integrity Of Primary Teeth After Radiation Therapy: An In-Vitro Study

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Abstract

Aim: To evaluate the effect of fluoride varnish on microhardness and structure of primary teeth after radiation.

Materials and Methods: Total thirty-six specimens of primary teeth were taken in this study, about twenty- four specimens were subjected to radiotherapy and twelve specimens were considered as control group. The specimens were divided into primary molars group (Group 1), irradiated primary molars group (Group 2) and irradiated fluoride varnish primary molars group (Group 3). The specimens from each group were subjected to microhardness, and scanning electron microscopy (SEM) analysis. The calcium and phosphate concentrations of artificial saliva was determined. The data was subjected to inter group comparison, which was done using one way ANOVA followed by pair wise comparison using post hoc test.

Results: This study showed that enamel surface hardness of irradiated fluoride varnish primary molars group (367.13± 58.0 kgf/mm2) was higher than that observed on primary molars group (328.4 ± 59.5 kgf/mm2). ($p<0.001$) The dentin surface hardness was highest in primary molars group (56.05 ± 5.5 kgf/mm2) and least in irradiated primary molars group (25.8 ± 4.2 kgf/mm2) ($p<0.001$). SEM images showed cracks on enamel and degradation of peritubular dentin in irradiated primary molars group.

Conclusion: The application of 5% sodium fluoride varnish during the irradiation treatment preserved the morphological and chemical integrity of the irradiated teeth.

Keywords: Dental enamel, Dentin, Fluorides, Radiotherapy

INTRODUCTION -

Every year, more than 5,50,000 cases of oral neoplasm are identified worldwide, with approximately 3,000 people dying as a result.[1] Lymphoma is the second commonest juvenile cancer in India, after brain tumors, with a reported prevalence of 12 to 25% of all childhood cancers.[2]. On the final and inoperable phases of disease, radiation was used as a main or adjuvant therapeutic treatment in some cases.[3]

Radiation-related caries, often known as "radiation caries," is a chronic side effect of head-and-neck irradiation often affects the cervical areas, incisal margins, and cusp regions of anterior and posterior teeth, with tooth surfaces obtaining a dark or brownish hue.[4] There are a variety of fluoride-containing professional products in the market, and their anti-cariogenic efficacy is determined by the product formed in the enamel and its long-term retention on the enamel surface.[5] Despite the lack of a consistent treatment plan for radiation-related caries, fluoride's importance is widely recognised. Regular use of 1% neutral sodium fluoride gel could reduce post-radiation caries.[6] However, less research has been conducted to investigate the effects of 5% sodium fluoride varnish on properties of enamel and dentin of primary teeth throughout the radiation treatment. Therefore, the current study focuses on the influence of fluoride varnish on the microhardness and structure of primary teeth following radiation.

MATERIALS AND METHODS

In this study, 36 freshly extracted sound primary molars were stored in thymol (0.1 M, pH 7.0). The specimens were divided equally into 3 groups; Primary molars (Group 1), Irradiated primary molars (Group 2), and Irradiated fluoride varnish primary molars (Group 3). Teeth with developmental abnormalities were ruled out. Teeth were washed with distilled and deionized water using ultrasonic cleaner machine. A diamond disc was used to cut teeth in order to detach the dental crown from the root.[7] These specimens were kept in artificial saliva and subjected to 12 daily fractions of 180 cGy radiation, resulting in a total dose of 2,160 cGy. The specimens were subjected to surface microhardness analysis. In addition, concentrations of calcium and phosphate of the artificial saliva were determined. At each experimental phase, 2 specimens were set apart for scanning electron microscopy (SEM) analysis. The results were compared among the experimental groups.

Fluoride Varnish application on primary molars:

5% Sodium fluoride varnish applied once a week for three weeks during radiotherapy, according to the guidelines of the American dental association.[8,9,10]

Primary molars irradiation:

The 180 cGy fractions were administered every day for 5 days, followed by a 2-day weekend break, and then another 5 days until a total of 12 daily fractions of 180 cGy of gamma radiation were administered on group 2 and group 3, resulting in a total dosage of 2160 cGy. This method was designed to mimic the radiation regimen used in pediatric Hodgkin's lymphoma.[11] Radiation doses were administered using the Varian- Clinac 6EX with a linear accelerator and 6 MeV beam photons to irradiate the specimens with a 99.9% margin of total dose, using the field of 15 x 15 cm, there was a focus of 100 cm.[12]

Specimen preparation:

After irradiation, crowns were then sectioned to separate the buccal and lingual surfaces, providing 3 x 3 x 2 mm specimens.[7] Sandpaper grains of 800 were used to polish enamel. Sandpaper grains of 1,200 were employed for dentin polishing.[12] After each sandpaper polishing, the specimens were cleaned for 2 minutes in an ultrasonic cleaner with distilled and deionized water.

Vicker's micro-hardness of study groups specimen:

The microhardness of Group 1, Group 2 and Group 3 was determined using Vicker's microhardness tester (Omni Tech, Piedmont, Model-S.Auto). A pyramid diamond indenter was used at three sites to apply a force of 50 kgf to the enamel surface and 25 kgf to the dentin surface for five seconds.[12] The surface hardness value was calculated using the mean average of three indentations made in a row at a spacing of 100 micrometers between them.

Calcium/Phosphate concentration of study groups specimen:

The calcium and phosphate concentrations were measured using a spectrophotometer that had been calibrated with a blank cuvette and a standard cuvette containing 10 mg/dL calcium and 5.0 mg/dL phosphate, respectively.[12]

SEM of study groups specimen:

The specimens were then inspected at 15 kV acceleration voltage using a scanning electron microscope (JEOL, JSM – 6360 LV, Tokyo, Japan). The enamel surfaces were imaged at 1,500 magnifications, whereas the dentin surfaces were imaged at 2,500 magnifications.

Statistical analysis

The data for Micro-hardness number, calcium and phosphate concentrations in artificial saliva were subjected to the inter group comparison was done using one way ANOVA followed by pair wise comparison using post hoc test. For these analyses, a 5% significance level was established. Statistical Product and Service Solution (SPSS) version 21 for Windows (Armonk, NY: IBM Corp) software was used to analyse the data.

RESULTS

For the enamel, the surface hardness of group 3 (367.13± 58.0 kgf/mm2) was significantly high ($p<0.001$) than that observed on group 1 (328.4 ± 59.5 kgf/mm2) and least in group 2 (279.56 ± 51.5 kgf/mm2). [**Graph 1**] The dentin surface hardness was highest ($p<0.001$) in group 1 (56.05 ± 5.5 kgf/mm2) followed by group 3 (41.68 ± 5.0 kgf/mm2) and least in group 2 (25.8 ± 4.2 kgf/mm2). [**Table 1**] The phosphate and calcium concentration in the artificial saliva increased progressively in group 2 (0.074 ± 0.02, 1.24 ± 0.05) as compared to group 3 (0.064 ± 0.07, 0.071 ± 0.03) and group 1 (0.05 ± 0.01, 0.52 ± 0.07). [**Table 2**]. The findings of the SEM study revealed significant structural and functional changes on enamel and dentin surfaces. Dentinal tubules were visible on dentin surfaces, however there were no fractures or outer surface discontinuity on enamel surfaces in the group 1. Group 2 had progressive obliteration of tubules on dentin surfaces, as well as surface fractures or outer surface discontinuities on enamel surfaces. Fluoride particles observed on the dentin surface of group 3 as approximately spherical globules seemed to merge and create a micro-structured surface layer to prevent obliteration of dentinal tubules. The enamel surface of group 3 had a small outer surface discontinuity but no surface fissures. [**Figure 1a, 1b, 1c**]

DISCUSSION

Patients who get radiation therapy for head and neck cancer have a significant risk of tooth decay.[12]. Preventive measures should be taken before, during, and after radiotherapy. Therefore, the current study focuses on the influence of fluoride varnish on the microhardness and structure of primary teeth following radiation. The current study's findings, as well as those of prior in vitro investigations, support this theory. The samples were put in artificial saliva, during irradiation; in the current study to mimic as closely as feasible the circumstances encountered in the mouth.[13] In comparable research, alternative media, such as Thymol solution, have been employed to preserve the teeth.[12]

Due to increasing number of cancer cases in paediatric patients, and because permanent teeth and primary teeth have different morphology, structure, and content, extracted primary molars were chosen for this study.[14,15] The surface microhardness was measured using Vickers micro hardness tester (Omni Tech, Model-S.Auto, Pune) as it is quick, easy, feasible and very simple to perform as compared to other techniques like polarized light microscopy, quantitative light-induced fluorescence (QLF), diagnodent, or FluoreCam.[16] In the present study, irradiation considerably reduced the surface microhardness of the enamel of group 2 following radiation doses (2160cGy) when compared group 1 and group 3. Furthermore, when compared to group 1 and group 2, the enamel of group 3 showed an increase in surface hardness. De Siqueira Mellara et al. (2014), on other hand, only detected an increase in surface hardness at 4,000 cGy of radiation.[17]. This study findings corroborate those of De Siqueira Mellara et al. (2014), who found poorer dentin microhardness after radiation when compared to baseline values.[17]

Lenita Marangoni-Lopes et al. (2019) concluded that fluoride promote reductions in phosphate and carbonate content were found after radiation doses.[12] Also, few studies have demonstrated morphological changes of dental hard tissues exposed to gamma radiation. SEM provides the best images for a quick evaluation of surface changes, showing features on a submicron scale.[18] In present study, group 2 showed surface fractures or outer surface discontinuities on enamel surfaces and obliteration of dentinal tubules on dentin surfaces after radiation doses (2160 cGy). Madrid et al. (2017) discovered abnormalities and a loss of enamel organic matrix in SEM images of transverse sections of irradiated permanent teeth following radiation doses ranging from 5,000 to 7,000 cGy.[19] These authors utilized doses that were 1.6 to 2.3 times more than those used in current study. The current study likewise identified alterations in primary enamel, despite the fact that, mineral and organic content of this substrate was lower than that of permanent teeth.[20]Sodium fluoride varnish had a significant effect in this study, in minimizing loss of hardness of enamel and dentin of group 3, by conducting the SEM analysis. This is owing to fluoride ions' capacity to replace hydroxyl ions in the formation of fluoroapatite, which is tougher and less prone to dissolution. Fluoroapatite reduces the amount of space occupied by the organic matrix, the component most impacted by gamma irradiation.[13] In previous literature, Lopes et al. (2018) conducted study using application of 1% neutral fluoride on third molars during radiotherapy and concluded that enamel irradiated with fluoride showed the lowest degree of demineralization.[9] The current study results are in agreement with (Meyerowitz et al. 1991), who reported that application of 0.05% sodium fluoride mouthwash two times a day for a period of 28 days which enhances remineralization of enamel subjected to therapeutic radiotherapy dose.[21] Furthermore, Markitziu and colleagues (1991) found that when irradiated enamel was treated with fluoride, the solubility of enamel decreased.[22] In comparison to group 2, the results of this investigation suggested that sodium fluoride varnish has a protective impact on group 3. This outcome is consistent with findings from Almqvist and Lagerlof (1993) who founded that fluoride concentrations in teeth after rinses with 0.025, 0.2, and 1.0% sodium fluoride are particularly effective in reducing root hard-tissue demineralization and increasing remineralization.[23] As a result, the use of fluoride during radiation therapy is significant in the endeavour to reduce treatment side effects on tooth enamel and enhance the quality of life of patients with head and neck tumours.

CONCLUSION

The application of 5% sodium fluoride varnish during the irradiation treatment preserved the morphological and chemical integrity of the irradiated teeth. Thus, preventive oral health care programmes using fluoride varnish and mouthwash should be part of the overall management of radiotherapy side effects.

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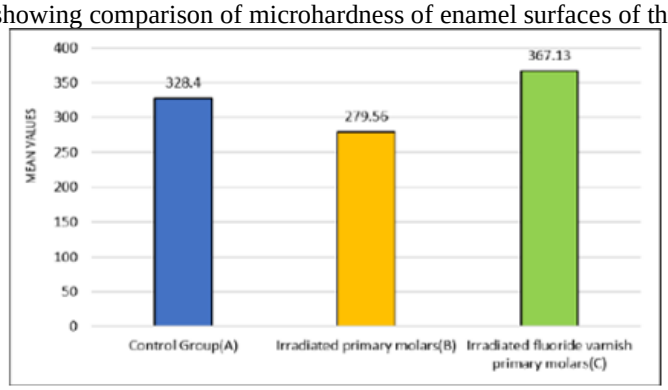
Table 1 Means and standard deviations of dentin surface microhardness values at different experimental group

Experimental groups	Dentin surfaces	P Value
Group 1	56.05 ± 5.5	p < 0.001**
Group 2	25.8 ± 4.2	
Group 3	41.68 ± 5.0	
p value <0.001 indicates significant difference.		

Table 2 Means and standard deviations of calcium and phosphate concentration values at different experimental group

Experimental groups	Calcium	phosphate	P Value
Group 1	0.52 ±0.07	0.05 ±0.01	p < 0.001**
Group 2	1.24 ±0.05	0.074 ±0.02	
Group 3	0.71 ±0.03	0.064 ±0.07	
p value <0.001 indicates significant difference.			

Graph 1 showing comparison of microhardness of enamel surfaces of three groups



Graph 1

Figure1 (1a) Scanning electron micrograph images of enamel surface (Control group), Arrows showing dentinal tubules. (1b) Scanning electron micrograph images of enamel surface (Irradiated Primary molars group). Arrows showing surface fractures and outer surface discontinuities. (1c) Scanning electron micrograph images of enamel surface (Irradiated Primary molars group with fluoride varnish). Arrows showing small outer surface discontinuity but no surface fracture.

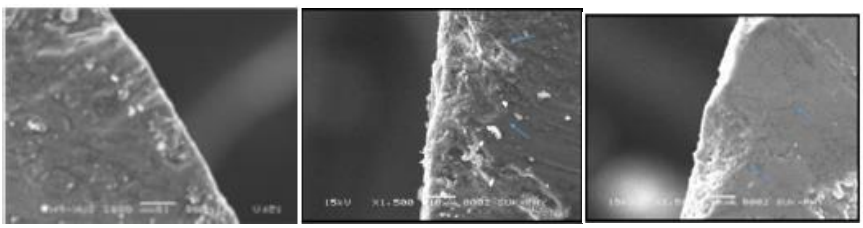


Fig (1a) **Fig (1b)** **Fig (c)**