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Effect of Diode Laser and Remineralizing Agents on Microstructure and Surface Microhardness of Therapeutic Gamma-Irradiated Primary Teeth Enamel

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Abstract

BACKGROUND: Radiation caries is a serious complication to head and neck cancer (HNC) radiotherapy, for which the primary teeth are more susceptible to be affected. Preventive protocols are recommended to enhance dental structure resistance against the direct effects of radiotherapy.

AIM: The aim of the study is to evaluate the effect of diode laser and two types of remineralizing agents on the microhardness of the primary teeth enamel and examine microstructural alterations.

METHODS: Twenty primary molars were sectioned into two halves in a mesiodistal direction, to obtain 40 specimens, which were then randomly allocated into five groups. Group 1 (Control Negative) n = 5 was not subjected to any treatment or radiation. Group 2 (Control positive) n = 5 was gamma irradiated with a dose of 60 Gray. For Groups 3, 4, and 5, specimens were divided into two subgroups: A and B (n = 5/subgroup). Subgroups A were gamma irradiated, then exposed to different surface treatments: 3A:10% nano-hydroxyapatite (nHA) paste, 4A: 5% sodium fluoride varnish (FV), and 5A: diode laser 980 nm. Subgroups B were exposed to surface treatments (3B: 10% nHA, 4 B: 5% FV, and 5B: diode laser 980 nm), then gamma irradiated. Surface micromorphology and microhardness were examined using environmental scanning electron microscope (ESEM), and Vickers microhardness tester, respectively.

RESULTS: Group 2 (G) specimens possessed the lowest mean microhardness, while nHA-G (3B), G-Fl (4A), and L-G (5B) had significantly higher values. ESEM analysis showed an alteration in Group G and the obliteration of enamel micropores with remineralizing agents. The melting and fusion of enamel in laser subgroups were also observed.

CONCLUSIONS: The findings indicated that using FV, nHA, or diode laser increased microhardness and maintained the integrity of the enamel microstructure. Therefore, applying preventive strategies should be considered in HNC radiotherapy.

Introduction

Head and neck cancer (HNC) represents almost 13.6% of new cancer cases annually, ranking seventh worldwide [1]. HNC includes a multiple group of tumors affecting the oral cavity, and nearby structures, for which radiotherapy is the most common treatment modality [2]. Nevertheless, it damages both the cancer and normal cells [3]. Radiation caries (RC) is one of the clinical sequences of radiotherapy that impair the patients' quality of life [4], as it damages the dental structures in a distinctive way and develops rapidly displaying a fast progression. Consequently, teeth become brownish, brittle, and liable to fracture [5], [6]. Radiotherapy induces caries in direct and indirect ways [5]. The direct effects of ionizing radiation on enamel include the alterations in the chemical and mechanical properties, and the increase in acid solubility of the enamel [7]. Following the cumulative irradiation with 30 and 60 Gray (Gy), some morphological alterations in the enamel were detected in the form of disorganization in the prismatic structures [8].

Managing RC is difficult for both dentists and patients, making it necessary to follow preventive and therapeutic strategies to avoid the high risk of RC in HNC patients [7]. As per the American Dental Association recommendations for high risk patients, fluoride varnish (FV) should be applied 2–4 times per year [9]. The nano-hydroxyapatite (nHA) were proposed for their anti-cariogenic properties, as an alternative to fluoride. Hence, they received a great attention in recent dental research for their direct impact in the repair of damaged enamel [10], [11]. Moreover, laser was proven to alter the permeability and crystalline structure, and advance the demineralization resistance of tooth enamel. A wide range of lasers have been used to improve the resistance of the tooth structure to caries [12]. The previous studies utilizing diode laser reported that the enamel surface of the primary teeth showed melting and re-solidification. As a result, diode laser may play a critical role in the prevention of dental caries [13].

To the author's best knowledge, no study had conducted a comparative investigation of the use of diode laser, nHA, and topical FV as preventive strategies against the damaging effect of gamma irradiation on the primary teeth enamel. The present study is motivated by the increase in HNC cases among children, and the few available reports on the preventive strategies for primary enamel. This *in vitro* study aims to evaluate the efficacy of diode laser, FV, and nHA on surface microhardness, and micromorphology of the primary teeth enamel, before and after exposure to radiotherapy.

Materials and Methods

Sample selection and preparation

In the present study, 20 primary molar teeth were collected according to the physiologic root resorption conditions. An informed consent was obtained from the children's parents. The collected teeth were visually examined. The inclusion criteria were specified as follows [14]:

- Caries free buccal and lingual surfaces
- No structural defects, no visible cracks, and no previous dental restoration
- Teeth unexposed to radiation, and not subjected to pretreatment with chemical agents, such as hydrogen peroxide [15].

The collected teeth were cleaned under running water with a soft brush to remove any residual tissues and debris. The teeth were polished with non-fluoride polishing paste, and brushed by air motor (W&H, Austria), then kept in a glass container filled with distilled water for no more than 1 month, at room temperature to avoid dehydration, and replenished daily [16], [17]. One clinician prepared all the teeth. Crowns were divided in the mesiodistal direction into buccal and lingual halves, using a dental diamond disk (DFS, Diamond, Germany) in a low-speed handpiece and micromotor [14], yielding 40 samples that were then placed individually on top of a freshly mixed cold-cured acrylic resin (Acrostone, Egypt) made in prepared molds. Each buccal and lingual surface were mounted centrally in the acrylic resin, facing upward for enamel surface treatments.

Teeth samples grouping

The 40 samples were coded, and randomly allocated into five groups, as presented in Table 1, and Groups 3–5 were further divided into two subgroups, of five specimens each.

Table 1: Grouping of specimens

Group	Code	Surface treatment applied
1	C	Control, only stored in distilled water
2	G	Only received gamma irradiation (60 Gy)
3		
3a	G-nHA	Gamma irradiated, then treated with experimental nHA paste (10%)
3b	nHA-G	Treated with experimental nHA paste, then gamma irradiated
4		
4a	G-Fl	Gamma irradiated, then treated with fluoride varnish
4b	Fl-G	Treated with fluoride varnish, then gamma irradiated
5		
5a	G-L	Gamma irradiated, then treated with diode laser
5b	L-G	Treated with diode laser, then gamma irradiated

nHA: Nano-hydroxyapatite.

Materials applied

Table 2 details the materials used in this study.

Table 2: Remineralizing agents used, with their composition and manufacturer

Material	Composition	Manufacturer
Experimental nHA white paste (10%)	Needles shape hydroxyapatite particles with length = 120 nm ± 30 nm, width = 20 + nm 10Ca (OH) 2 + 6H3PO4 → Cal0(PO4) 6(OH) 2 + 18H20	Nano Gate, Egypt www.nanogate-eg.com
3M™ Clinpro™ 5% sodium fluoride white varnish	Tri-calcium phosphate, an alcohol-based solution of modified rosin, sodium fluoride, and xylitol	3M ESPE, USA www. 3mespe.co.uk Lot no.NC83141

nHA: Nano-hydroxyapatite.

Experimental nHA paste application

The enamel specimens were dried with gauze, and the nHA white paste (10%) was applied in a copious amount, using a micro-brush to cover the whole surface. The applied paste was left undisturbed in place for 30 min, then rinsed thoroughly with distilled water for 1 min to remove any visible paste remnants [16]. This application was repeated 7 successive times for a total of 3.5 h [18].

FV application

3M™ Clinpro™ 5% sodium fluoride white varnish was applied to the enamel specimens, after drying them with a gauze. A thin layer was evenly applied with a sweeping brush in horizontal strokes. The applied layer was left undisturbed in place for 24 h, in distilled water at room temperature [19], then wiped off with a sterile gauze, and a low-speed brush to simulate tooth brushing [20].

Diode laser irradiation

Windows of 3 mm × 3 mm were made to standardize the area of all specimens in Groups G-L and L-G that were to be exposed to laser irradiation. The periphery of the windows was coated with nail varnish [21]**.** The enamel specimens were irradiated in the National Institute of Laser-Enhanced Sciences, Egypt, using a diode laser device (Quanta c 980 nm, Italy) at 2 watt for 15 sec, in a continuous and contact modes, using an optic fiber transmission system with a diameter of 320 µm. The fiber tip was manually held perpendicular to the surface and moved longitudinally in a uniform scanning motion over the entire exposed surface [12], [16], [22], [23].

Sample exposure to therapeutic gamma radiation

This step was executed in the National Center for Radiation Research and Technology, at the Atomic Energy Authority (NCRRT), Cairo, Egypt, using (60 co, Baha, Baha Indian Cobalt: 60–4000A) as a gamma source, at a dose rate of 0.806 KGy/h at the time of irradiation. A simulated therapeutic dose of 60 Gy was administered as a single dose [24], which is the therapeutic dose of HNC patients [25], [26]. All enamel specimens – except those of Group C – were exposed to therapeutic gamma irradiation, while immersed in plastic containers filled with distilled water [27].

Assessment methods

Surface micromorphology examination

Representatives from the specimens of each group were examined by an Environmental Scanning Electron Microscope (Model: Quanta FEG-250, Netherlands), with magnification of X2,000, to observe the micromorphological alterations. No special preparations were required before the examination.

Surface microhardness testing

The specimens' surface microhardness was determined using digital Vickers hardness tester (INNOVATEST, model no.4503, Netherlands), with magnification ×40, and a Vickers diamond indenter that has a square base and pyramid shape, to enable penetration into the enamel surface. The best point for load application was determined, and a single load of 100 g was applied for 10 sec [19], [22]. Three randomized indentations were made on the enamel surface. Surface microhardness calculations were conducted using universal testing machine (Tension and compression SHIMADZU 5 KN (AUTOGRAPH AG_ X PLUS), Japan). The average of all three Vickers hardness values obtained from the enamel was recorded and expressed as VHN (g/mm^2).

Statistical analysis

Numerical data were explored for normality by checking the data distribution using Kolmogorov–Smirnov and Shapiro–Wilk tests. Data showed parametric distribution; thus, they were represented by mean and standard deviation (SD) values. One-way ANOVA followed by Tukey's *post hoc* test were used to analyze different intergroup comparisons. The significance level was set at $p \leq 0.05$ within all tests. The statistical analysis was performed with *R* statistical analysis software version 4.1.2 for Windows.

Results

ESEM analysis

Under magnification X2,000 (Group C), a typical pattern of enamel prisms and interprismatic structure was detected on sound enamel surface of primary teeth (Figure 1a), whereas in irradiated enamel (Group G), a merging of both structures was observed, showing distorted prisms with an apparent morphological alteration (Figure 1b).

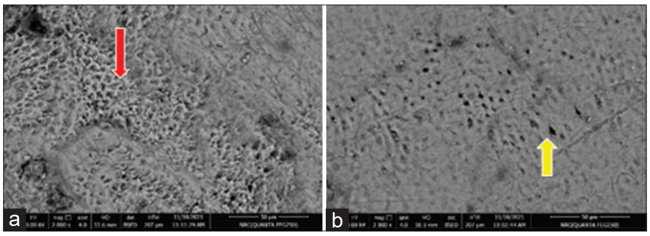

Figure 1: Environmental scanning electron microscope micrographs: (a) Group C showing normal enamel prisms and inter-prismatic structure (red arrow) and (b) Group G showing distortion of enamel prisms with merging of prismatic and interprismatic structures (yellow arrow) (×2000)

Gamma irradiation before surface treatments

Obscured micro-porosities were observed in Group G-nHA, in addition to a few small furrows and scratches (Figure 2a), whereas in Group G-FI, an almost smooth surface was created by areas of crystallized deposits, concentrated along the peripheries of enamel prisms with obvious obliteration of the surface micropores (Figure 2b). By observing G-L specimen, fused and melted parts were identified with shallow furrows, and traces of prismatic structure (Figure 2c).

Gamma irradiation after surface treatments

Group nHA-G revealed multiple scattered distorted micropores, while others were blocked or diminished in size, with few microcracks (Figure 3a). Group FI-G showed similar findings to the previously mentioned Group G-FI (Figure 3b). The irradiated enamel surface in Group L-G had a typical melting appearance with recrystallized enamel structure, and enamel globules of various sizes, generating an uneven surface with unclearly recognized enamel prismatic structures (Figure 3c).

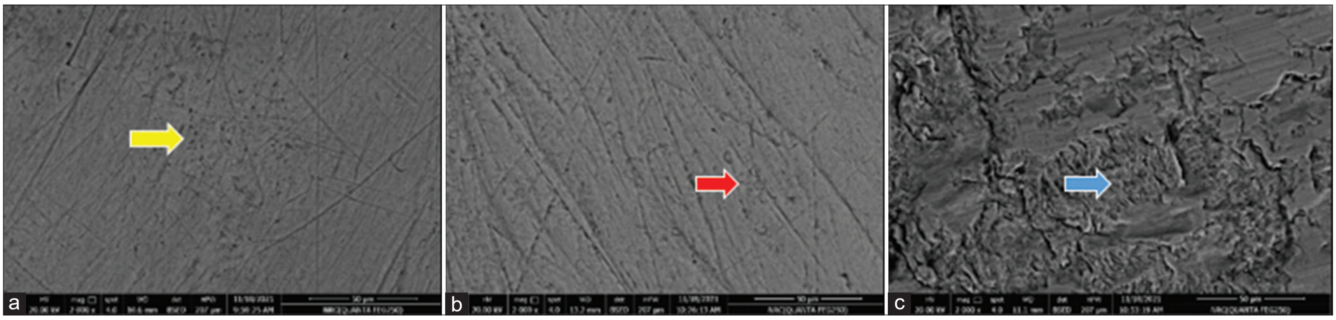

Figure 2: Environmental scanning electron microscope micrographs: (a) subgroup G-nHA showing obscured micro-porosities (yellow arrow) with superficial scratches; (b) subgroup G-Fl showing deposits concentrated along the peripheries of enamel prisms, with obvious obliteration of the surface micropores (red arrow) and superficial scratches; and (c) subgroup G-L showing fused and melted parts (blue arrow) (×2000)

Vickers hardness testing

Table 3 and Figure 4 show the mean and SD values of microhardness for different groups. Results revealed that the gamma-irradiated enamel specimens recorded the lowest mean microhardness values, whereas the highest value was obtained in G-Fl (392.43 ± 19.55). There was an increase in microhardness values among the experimental groups. On the other hand, *post hoc* pairwise comparisons showed that groups nHA-G, G-Fl, and L-G had significantly higher values than other groups ($p < 0.001$).

Discussion

Radiotherapy is one of the main methods for treating of HNC. Rather than the damage caused by xerostomia and its consequences, this *in vitro* study was concerned with the direct effects of radiation on tooth enamel, including the alterations in crystal composition, the increase in enamel solubility, and the decrease in microhardness. Yet, the mechanism of RC had not been precisely described. In this study, a single radiation dose of 60 Gy was used [24], which is the maximum cumulative dose provided to children suffering from HNC. In clinical practice, the fractions were given for 6 weeks, 5 days/week [26], [28]. The literature shows that fractionated doses are used to avoid damage in the oral soft tissues. Thus, in the present *in vitro* study, the single dose of 60 Gy was not clinically applicable, but showed the probable effect of one high dose on enamel [29]. Moreover, the samples in the present study were placed in distilled water to avoid dehydration, which affects the mechanical properties of the tooth structure [30]. Primary teeth were selected due to the increased prevalence of cancer cases among children [8]. Primary and permanent teeth show different morphology, structure, and composition, since the organic content of the primary tooth enamel is higher than that of permanent tooth enamel. Hence, primary tooth enamel may be more susceptible to RC [31].

The direct effects of radiation were assessed through changes in the mechanical properties, and micromorphology using Vickers microhardness test and environmental scanning electron microscope (ESEM), respectively. Although the enamel is mainly inorganic, studies suggested that the early radiation damages occurred in the organic region of the enamel, which is the interprismatic area, where concentrated water molecules oxidized to hydrogen peroxide, and hydrogen free radicals, causing the denaturation of the organic components, and contributing to dental problems post head and neck radiation therapy [26], [32]. Another study found that inorganic components were also affected, and appeared as microcracks in the hydroxyapatite crystals [33]. This was demonstrated in the present study by the ESEM, as the irradiated sample showed a

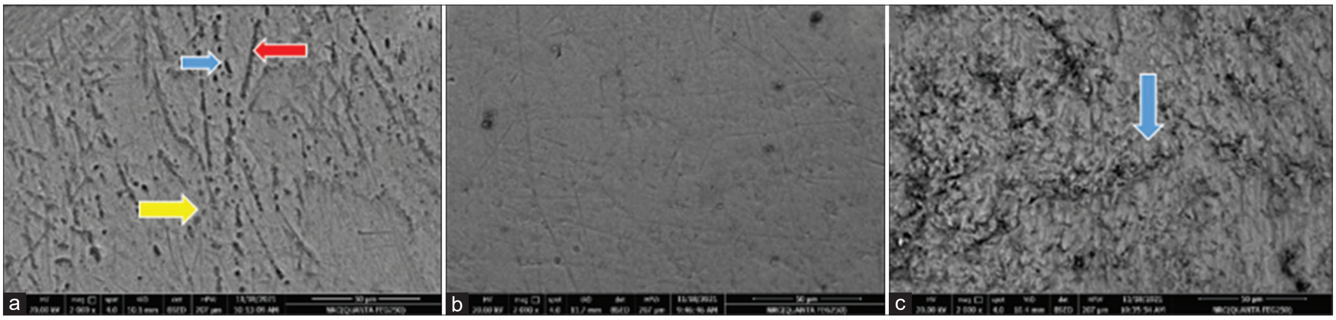

Figure 3: Environmental scanning electron microscope micrographs: (a) subgroup nano-hydroxyapatite-G showing multiple scattered distorted micropores (blue arrow), while others were blocked or diminished in size (yellow arrow), with few microcracks (red arrow); (b) subgroup Fl-G showing an almost smooth surface with obvious obliteration of the surface micropores; and (c) subgroup L-G showing enamel globules of various sizes (blue arrow), with unclearly recognized enamel prismatic structures (×2000)

Table 3: Mean, standard deviation, and results of the statistical analysis of microhardness values for different groups

Microhardness (mean ± SD)								p
C	G	G-nHA	nHA-G	G-Fl	Fl-G	G-L	L-G	
325.80 ± 14.06^{B}	306.83 ± 1.61^{B}	318.13 ± 18.37^{B}	386.27 ± 19.79^{A}	392.43 ± 19.55^{A}	312.77 ± 7.43^{B}	328.83 ± 20.85^{B}	379.50 ± 20.54^{A}	<0.001*

*Significant ($p \leq 0.05$). Means with different superscript letters are statistically significantly different. SD: Standard deviation, nHA: Nano-hydroxyapatite.

merging of both prismatic and interprismatic structures, with distorted prismatic peripheries. This is in agreement with findings by Duruk *et al*. [14], Bakr [21] and Rodrigues *et al*. [31]. Furthermore, this change in the enamel crystalline structure was suggested as one of the factors related to the increased risk of dental caries following radiation therapy. Therefore, it was necessary to follow preventive and therapeutic strategies to avoid the damaging effect of the gamma radiation, and the high risk of RC in HNC patient [7].

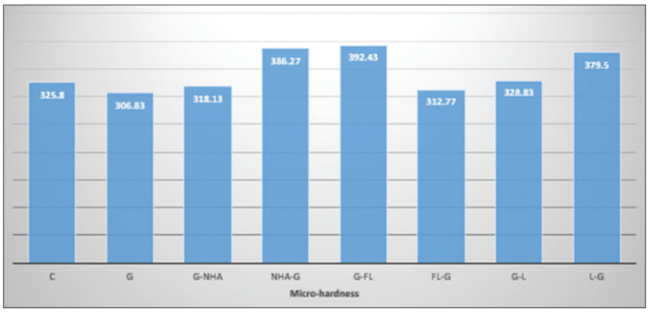

Figure 4: Bar chart showing average microhardness for different groups

The used procedure for the application of nHA paste simulated the use of the remineralizing agent for 5 min daily for 6 weeks, as it was found that remineralization took place in the first 3 to 6 weeks [34]. Huang *et al*. [10] concluded that the 10% nHAP, as used in this study, was the most effective concentration in the remineralization process. nHA have a high capacity in filling micropores and depressions due to the small size of the particles that compose it. It creates a smooth superficial layer on the enamel surface and yields a uniform crystalline enamel structure that enhances enamel resistance to caries [35], [36]**.** This was demonstrated in this study, as micropores were nearly blocked when the enamel was treated with experimental nHA paste, after being gamma irradiated. Roveri *et al*. [37] explained that the application of nHA crystals with size of 100 nm produced a homogeneous coating that hid interprismatic and prismatic enamel structures when examined with ESEM. On the other hand, some superficial cracks were found in the subgroup nHA-G, possibly due to the dehydration induced by gamma radiation [38].

FV application decreased the incidence of RC. Other topical fluoride forms are available, such as gel and foam. Nonetheless, varnish is the recommended form of topical fluoride application for children under 6 years of age [39]. In the present study, the varnish was left undisturbed for 24 h, because fluoride and inorganic ions were absorbed into the surface 24 h after application; so, immediate removal was not recommended [19]. A previous study revealed that topical fluoride application formed insoluble globules of calcium fluoride-like material on the tooth surface, where these globules act as a reservoir of fluoride [40]**.** This conclusion is in good agreement with the findings of the present study. In the study, the obliteration of the surface micropores was observed, creating a smooth enamel surface similar to the smooth and uniform surface found by Shihabi *et al*. [41]. These results could be attributed to the release of fluoride ions, leading to the formation of fluorapatite crystals instead of the hydroxyl ions in enamel, which are less susceptible to dissolution.

Diode laser 980 nm was used in this study for its ability to increase enamel resistance to caries, reduce solubility in acids, and increase surface microhardness, as proven in previous studies [12], [22]. Besides, the low cost, small size, and ease of use in the oral cavity made diode laser attainable. Although diode laser is known for soft tissue applications, its use in hard tissue application can be justified based on the findings by Romanos and Nentwig [42], who reported that the penetration depth of a diode laser at 980 nm wavelength was smaller than that of erbium and Nd: YAG laser. Increased energy deposition at the surface, melting, and recrystallization of the enamel structure occurred as a result of the shallower penetration depth. The laser parameters used in the present study were adopted from the previous studies [16], [22], which stated that laser light must be properly absorbed and transformed into heat without injury to underlying tissues. The results of the ESEM analysis of both laser subgroups showed areas of melting and fusion and revealed recrystallized enamel structures with the appearance of globular crystals of different sizes, generating an uneven surface with unclearly recognized enamel prismatic structures. The likely justification, provided by Pavithra *et al*. [22], was the release of intercrystalline substance, obstructing interprismatic spaces, increasing the resistance of the dental enamel against acids, and decreasing enamel permeability [17]. Nevertheless, the results of the present study did not correspond to the findings by Santaella *et al.* [43], since the treated areas showed no morphological alterations. This may be attributed to the different laser parameters; 809 nm diode laser used in their study. The laser parameters governed the depth of penetration and temperature elevation and accordingly determined the chemical or morphological changes to dental tissues [44].

The VHN results showed that microhardness of gamma-irradiated enamel decreased insignificantly, which agrees with the findings by Duruk *et al*. [14], and Klarić Sever *et al*. [29], yet differs from results in other studies [8], [25] that recorded an increase in surface microhardness with cumulative doses higher than

30 Gy. The crystal structure of the mineralized tissues may be subjected to some structural changes due to the ionizing radiation, which can modify their physical properties, including the microhardness. The reduced microhardness could decrease tooth deformation during mastication, enabling enamel delamination few months post-radiation therapy [45]. Furthermore, the results of the present study revealed that all surface treatments induced an increase in the microhardness of gamma-irradiated primary teeth enamel compared to the gamma-irradiated control group. FV application enhanced the resistance of the sound enamel of primary teeth and showed significantly greater effect than other treatments in the post irradiation group, which agrees with the results by Huang *et al*. [10]. This could be attributed to the high fluoride content, allowing for more enamel fluoride uptake. Fluoride ion improved the development of enamel crystals and altered hydroxyapatite crystals into fluorapatite, reducing the space occupied by the organic matrix [46]. These findings emphasize the importance of fluoride in maintaining the integrity of enamel during and after radiotherapy. The increase in VHN observed in this study with the application of 10% nHA paste is in agreement with Huang *et al*. [10], and Kim *et al*. [47]. The penetration of the nHA into the enamel pores occupied the empty spaces among the enamel crystals, which could promote crystal integrity and growth. A former study [48] found that the nHA showed higher microhardness results than FV, which does not conform with the present results, possibly due to the different formulation, concentration, and time of application.

Despite the increase in VHN in both laser groups, there was a significant difference in L-G subgroup, for which microhardness values increased with diode laser irradiation before gamma radiation. This is in line with the previous studies [22], [49] that reported an increase in the microhardness of enamel when diode laser was applied, possibly due to the heat deposited at the exposed site, which discharged water, organic compounds, and carbonate ions from the hydroxyapatite structure, and enhanced the rate of mineralization and recrystallization [49]. On the other hand, Kato [50] reported the inefficiency of diode laser alone in decreasing the enamel solubility. The VHN obtained from enamel treated with materials containing fluoride (FV), or calcium and phosphate (nHA) was higher than that obtained from using diode laser alone, which is similar to the findings by Santaella *et al*. [43]. Finally, the calculated VHN in this study was comparable to that in the previous studies [48], [49], whereas somehow different from that reported in [14]. VHN variations can be attributed to the preparation of specimens, diagonal length reading error, and study design. The different indentation load and dwelling time should also be considered [51]. In this *in vitro* study, both salivary pH alteration and dietary factors in the oral cavity of the irradiated patients were not simulated to examine the combined effect of indirect and direct gamma radiation, with the application of different surface treatment strategies. Besides, the relatively small sample size could have influenced the outcome. *In vivo* studies are recommended to assess the efficacy of different preventive strategies against the combined direct and indirect effects of radiotherapy.

Conclusion

The findings of the present study revealed that applying different remineralizing strategies pre- or post-radiation reduced the damaging effect on the dental enamel in HNC patients. It can be concluded that the different preventive strategies should be considered before, during, or after radiotherapy for HNC patients, to reduce the potential damage of its direct effect. The findings also indicated that applying either FV or nHA increased the microhardness and maintained the integrity of the enamel microstructure. Diode laser could enhance enamel remineralization.

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