Original Article

Assessment of Microhardness of Demineralized Gamma-irradiated Human Enamel after Diode Laser and Fluoride Surface Treatment: A Randomized Controlled Trial

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ABSTRACT

Introduction: The physical and chemical alterations induced by laser irradiation hold the potential to enhance enamel resistance against demineralization. Particularly, the combination of diode laser therapy with topical fluoride application presents an innovative approach toward caries prevention and immediate fluoridation of teeth. The aim of this study is to evaluate the microhardness of demineralized gamma-irradiated human enamel following diode laser (980 nm) and fluoride surface treatment. **Materials and Methods:** The enamel samples of 18 primary anterior teeth were subjected to gamma radiation at a dose of 60 Gy, administered at a rate of 2 Gy per day for 5 days a week over a span of 6 weeks. The samples were then immersed in a demineralizing solution with a pH buffer of 4.01 for a period of 96 h. They were then divided into three groups – control group (did not receive any treatment), laser group (diode laser 980 nm), and sodium fluoride varnish + laser group. Postintervention, the microhardness of the enamel samples was determined using Vickers's test after demineralization and remineralization, respectively. **Results:** A statistically highly significant difference ($P < 0.001$) was noted when comparing the posttreatment microhardness values with the pretreatment values for both groups, laser and fluoride varnish, respectively. The pair-wise comparison revealed significantly higher postremineralization microhardness values for the samples in the order Group 3 > Group 2 > Group 1. **Conclusion:** The findings of the present study underscore the potential of combined diode laser therapy and fluoride varnish application in enhancing enamel microhardness and resistance to demineralization in gamma-irradiated enamel samples.

Keywords: *Dental caries, enamel microhardness, radiation, remineralization*

Clinical Relevance to Interdisciplinary Dentistry

The study highlights the potential for integrating combined diode laser and fluoride varnish treatment into dental care protocols for oncology patients, enhancing enamel resistance to radiation-induced caries. The findings encourage interdisciplinary collaboration between dental professionals and radiation oncologists to improve oral health outcomes in patients undergoing head and neck radiotherapy.

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Introduction

Dental caries remain a prevalent concern among individuals undergoing radiotherapy in the head-and-neck region, significantly impacting their quality of life.[1] The distinctive nature of postradiation dental lesions, characterized by their clinical presentation, developmental pattern, and progression,

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sets them apart from conventional dental decay observed in nonirradiated patients.[2] While typical dental caries tend to manifest in pits, fissures, and interproximal regions, postradiation dental lesions often occur at cervical, cuspal, and incisal areas, posing unique challenges in their management.

The abrupt onset and rapid progression of radiation-induced caries underscore the urgency of addressing preventive strategies, as these lesions could lead to extensive dental damage within the first year postirradiation.[3] The incidence of head-and-neck tumors, though relatively rare in children, is on the rise, with approximately 550 children affected annually in India.[4] Lymphoma, rhabdomyosarcoma, and thyroid cancers are among the most common malignancies in this region.

Radiation-induced hyposalivation stands out as a key etiological factor contributing to caries development postradiotherapy.[5] However, it is imperative to recognize the multifactorial nature of radiation decay, which involves not only reduced salivary flow but also alterations in saliva composition, pH levels, bacterial flora, dietary habits, and oral hygiene practices.[2,6]

Beyond caries, therapeutic radiation doses also give rise to complications such as xerostomia and mucositis, further exacerbating oral health challenges in affected individuals.[5,7] In this context, fluoride emerges as a cornerstone in caries prevention, with its ability to enhance enamel acid resistance, promote lesion remineralization, and inhibit the metabolic processes of cariogenic microorganisms.

Recent attention has been directed toward laser surface treatment of dental enamel as a promising avenue for caries prevention.[8] The physical and chemical alterations induced by laser irradiation hold the potential to enhance enamel resistance against demineralization. Particularly, the combination of diode laser therapy with topical fluoride application presents an effective synergistic approach toward caries prevention and immediate fluoridation of teeth.[9]

Hence, the aim of the present study is to evaluate the microhardness of demineralized gamma-irradiated human enamel following diode laser (980 nm) and fluoride surface treatment. By elucidating the efficacy of this combined intervention, we aim to contribute to the development of effective preventive strategies against radiation-induced dental complications, thereby improving the oral health outcomes and overall well-being of individuals undergoing head-and-neck radiotherapy.

Materials and Methods

The present *in vitro* study adhered to ethical guidelines and obtained approval from the institutional review board.

Sample preparation

Eighteen over-retained noncarious primary anterior teeth with intact crowns were collected and extracted. Enamel slabs measuring 4 mm × 4 mm were prepared from these teeth and mounted onto acrylic blocks to facilitate handling during experimentation.

Gamma radiation exposure

The enamel samples were subjected to gamma radiation at a dose of 60 Gy, administered at a rate of 2 Gy per day for 5 days a week over a span of 6 weeks. This procedure simulated the radiation regimen typically used in head-and-neck cancer therapy. The radiation exposure was carried out in the Department of Radiology, MY Hospital, MGM Medical College, Indore (MP).

Demineralization protocol

Following gamma radiation exposure, the enamel samples were immersed in a demineralizing solution with a pH buffer of 4.01 for a period of 96 h. This process induced controlled demineralization, mimicking the conditions conducive to caries development postradiotherapy.

Microhardness assessment

Microhardness of the enamel samples was determined using Vickers's test. Prior to any intervention, baseline microhardness measurements were obtained for all samples to establish a uniform starting point.

Group allocation and interventions

The demineralized enamel samples were randomly divided into three groups:

- Group A (control): This group served as the control and did not receive any additional treatment beyond demineralization
- Group B (laser): Enamel samples in this group underwent diode laser (Epic X, Biolase, Foothill Ranch, CA, USA) treatment using a 980 nm wavelength laser operating at 1 W power in continuous contact mode. The laser was applied to the enamel surfaces according to a standardized protocol
- Group C (fluoride varnish + laser): Enamel samples in this group were treated with sodium fluoride varnish (Fluoritop varnish) applied using a microbrush. Subsequently, diode laser treatment identical to that in Group B was administered.

Figure 1 depicts the intervention procedures being applied to the prepared samples.

Microhardness evaluation postintervention

Following the respective interventions, microhardness measurements were taken again for all samples using the Vickers test. This postintervention assessment allowed for the evaluation of any changes in enamel microhardness induced by the different treatment modalities.

Statistical analysis

Upon completion of data collection, all data entries underwent meticulous proofreading to ensure accuracy. The collected data were then organized and compiled in a Microsoft Office Excel Sheet (version 2019, Microsoft Redmond Campus, Redmond, Washington, United States). Statistical analysis was conducted using the Statistical Package for the Social Sciences (SPSS, version 26.0, IBM. IBM Corp. Released 2019. Armonk, NY: IBM Corp., United States). Descriptive statistics including frequencies and percentages for categorical variables, as well as means and standard deviations for numerical variables, were calculated to provide a comprehensive overview of the data. Prior to conducting inferential analyses, the normality of numerical data distribution was assessed using the Shapiro–Wilk test, revealing adherence to a normal distribution curve. Accordingly, parametric tests were employed for subsequent comparisons. Inter-group comparisons involving more than two groups were performed using one-way analysis of variance, followed by pair-wise comparisons using appropriate *post hoc* tests. Intra-group comparisons, involving only two observations, were conducted using paired *t*-tests. Significance levels were set at $P < 0.05$ for all statistical tests, with an alpha error (Type I error) of 5% and a beta error (Type II error) of 20%, thus ensuring a study power of 80%. This rigorous statistical approach facilitated robust analysis of the data and provided meaningful insights into the effectiveness of the interventions under investigation.

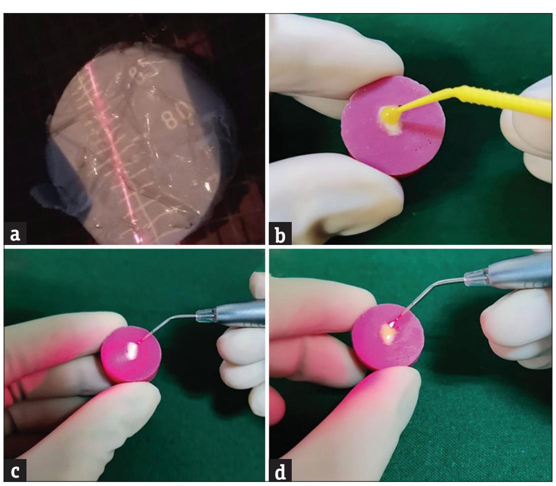

Figure 1: (a) Gamma irradiation of the sample; (b) Varnish application; (c) Laser application; and (d) Laser application to the varnish-treated sample

Results

The mean values before and after demineralization in all three groups are depicted in Figure 2. A statistically highly significant difference ($P < 0.001$) was noted when comparing the posttreatment microhardness values with the pretreatment values for both groups, laser and fluoride varnish, respectively [Table 1]. Inter-group comparison of the microhardness values showed that there was a statistically nonsignificant difference seen for the values between the groups ($P > 0.05$) at baseline, indicating that the baseline microhardness values following demineralization were identical [Tables 2 and 3].

Intra-group comparison of the mean microhardness values revealed a statistically highly significant difference in the microhardness values between all groups ($P < 0.001$) at the postremineralization interval. The difference in the microhardness values before and after remineralization was significantly greater for Group 3 as compared to Group 2.

The pair-wise comparison between groups revealed significantly higher postremineralization microhardness values for the samples in the order Group 3 > Group 2 > Group 1 [Table 3].

Discussion

Caries management in individuals undergoing head-and-neck radiotherapy remains a significant clinical challenge, despite the well-established role of fluoride in combating dental decay. While fluoride therapy has shown efficacy in nonirradiated populations, establishing a globally accepted protocol for radiation-induced caries remains elusive. Recent advancements in dental technology have explored the potential synergistic

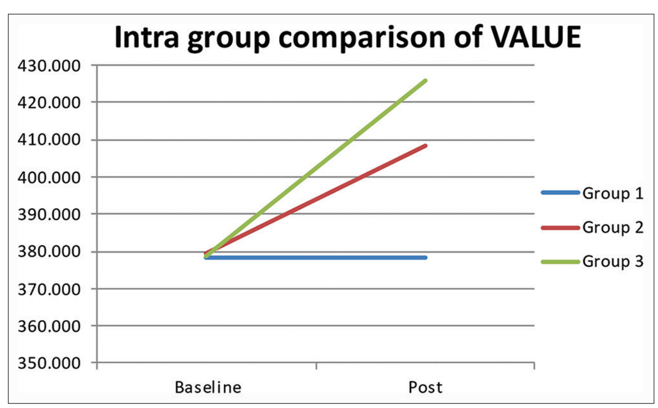

Figure 2: Change in the microhardness values of all groups before and after remineralization

Table 1: Intra-group comparison of the microhardness values before and after remineralization

Group	Interval	Mean microhardness (HV)	SD	SEM	Mean difference	SD of difference	*t*	*P* value of paired *t*-test
Control	Baseline	378.50	6.979	2.849	0	0	0	0
	Post	378.50	6.979	2.849				
Laser	Baseline	379.50	6.863	2.802	−29.000	8.832	−8.043	0.000**
	Post	408.50	3.834	1.565				
Fluoride varnish + laser	Baseline	378.83	6.210	2.535	−47.167	7.834	−14.748	0.000**
	Post	426.00	4.940	2.017				

**Statistically highly significant ($P<0.001$). SD=Standard deviation, SEM=Standard error of the mean, ANOVA=Analysis of variance, HV=Hardness Value

Table 2: Inter-group comparison of microhardness values for laser and fluoride varnish groups, respectively

Groups	Mean	SD	SEM	95% lower bound	95% upper bound	Minimum	Maximum	*F*	*P* value of one-way ANOVA
Baseline									
1	378.50	6.979	2.849	371.18	385.82	370	390	0.035	0.966#
2	379.50	6.863	2.802	372.30	386.70	371	388		
3	378.83	6.210	2.535	372.32	385.35	370	386		
Post									
1	378.50	6.979	2.849	371.18	385.82	370	390	118.309	0.000**
2	408.50	3.834	1.565	404.48	412.52	402	413		
3	426.00	4.940	2.017	420.82	431.18	419	432		
Difference									
1	0.00	0.000	0.000	0.00	0.00	0	0	73.096	0.000**
2	29.00	8.832	3.606	19.73	38.27	14	39		
3	47.17	7.834	3.198	38.95	55.39	38	55		

** = statistically highly significant, # = statistically non-significant. 1=Control, 2=Laser, 3=Fluoride varnish+laser, 95% CI for mean. SD=Standard deviation, SEM=Standard error of the mean, CI=Confidence interval, ANOVA=Analysis of variance

Table 3: Inter-group pair-wise comparison using Tukey's honestly significant difference *post hoc* tests

Dependent variable	(I) Group	(J) Group	Mean difference (I−J)	SE	*P*	95% CI	
						Lower bound	Upper bound
Baseline	1	2	−1.000	3.864	0.964#	−11.04	9.04
		3	−0.333	3.864	0.996#	−10.37	9.70
	2	3	0.667	3.864	0.984#	−9.37	10.70
Post	1	2	−30.000*	3.123	0.000**	−38.11	−21.89
		3	−47.500*	3.123	0.000**	−55.61	−39.39
	2	3	−17.500*	3.123	0.000**	−25.61	−9.39
Difference	1	2	−29.000*	3.935	0.000**	−39.22	−18.78
		3	−47.167*	3.935	0.000**	−57.39	−36.95
	2	3	−18.167*	3.935	0.001**	−28.39	−7.95

* = statistically significant, ** = statistically highly significant, # = statistically non-significant SE=Standard error, CI=Confidence interval

effects of combining diode laser therapy with topical fluoridation, presenting a novel approach to enhance fluoride binding to enamel surfaces.[9]

The present study investigated the efficacy of diode laser therapy combined with fluoride varnish application in enhancing enamel microhardness in gamma-irradiated enamel samples. Analysis of inter-group comparisons revealed statistically nonsignificant differences in microhardness values between the groups at baseline assessment. These findings suggest that, initially, there were no inherent differences in enamel microhardness among the treatment groups, highlighting the homogeneity of the samples before intervention.

A significant increase in the microhardness values was observed following treatment with laser alone and in combination with fluoride varnish. The increase in microhardness values was higher in the latter. These results indicate that both laser therapy alone and in combination with fluoride varnish application effectively enhanced enamel microhardness following demineralization induced by gamma radiation. Pair-wise comparisons revealed the combination of fluoride varnish and laser to be more efficacious in increasing the microhardness values as compared to lasers alone.

The observed improvements in enamel microhardness posttreatment can be attributed to several factors. Diode lasers, especially those emitting at a wavelength of 980 nm, can enhance enamel hardness by inducing localized melting and re-crystallization of enamel hydroxyapatite crystals. When the laser energy is applied to the enamel surface, it generates heat that causes the surface layer to partially melt. Upon cooling, this melted layer re-crystallizes into a more organized and dense structure, which is less susceptible to acid dissolution and more resistant to mechanical forces. This process not only repairs minor surface defects but also promotes the formation of a harder, more resilient enamel surface. In addition, the heat generated by the laser can help to remove organic materials and precipitate minerals, contributing to a more mineralized and durable enamel layer, effectively reducing the potential for future demineralization and decay.[10]

Inter-group comparisons also revealed that the combined application of sodium fluoride varnish and diode lasers was superior in improving the microhardness of the enamel as compared to lasers alone. These findings indicate that the application of fluoride varnish prior to laser treatment may have augmented its effects by promoting fluoride incorporation into the enamel structure.[11,12] This synergistic action likely contributed to the observed increases in enamel microhardness, as fluoride ions play a crucial role in remineralizing enamel and enhancing its resistance to demineralization.

Furthermore, our results align with previous studies demonstrating the efficacy of laser therapy and fluoride application in enhancing enamel hardness and resistance to demineralization.[9,11,12] Pavithra *et al*. reported similar findings, showing that diode laser (980 nm) irradiation significantly increased the hardness of pit and fissure enamel.[13] An earlier scanning electron microscopy (SEM) analysis by Bakr *et al*. corroborated our findings, revealing distinct structural differences between gamma-irradiated enamel subjected to demineralization and those treated with laser therapy.[14,15] While gamma-irradiated enamel exhibited total loss of the prismatic structure and deep pores, laser-treated enamel displayed a confluence of prismatic and interprismatic structures with marked pitting.

However, it is important to acknowledge some limitations of our study. The sample size was relatively small, and the follow-up period was limited. Future studies with larger sample sizes and longer follow-up durations are warranted to validate our findings and assess the long-term efficacy of combined laser and fluoride therapy in managing radiation-induced caries. Future studies can also compare the effects of different types of lasers such as comparison of the 890 nm diode laser to the 2940 nm erbium-doped yttrium aluminum garnet laser or combinations with different types of fluoride such as stannous fluoride, acidulated phosphate fluoride, and sodium monofluorophosphate.

Overall, the findings of the present study contribute to the growing body of evidence supporting the use of combined laser and fluoride therapy as a promising approach for managing radiation-induced caries and improving oral health outcomes in this vulnerable population. Further research is warranted to optimize treatment protocols and assess long-term outcomes in individuals undergoing head-and-neck radiotherapy.

Conclusion

The findings of the present study underscore the potential of combined diode laser therapy and fluoride varnish application in enhancing enamel microhardness and resistance to demineralization in gamma-irradiated enamel samples. Through meticulous inter-group and intra-group comparisons, we have demonstrated the efficacy of this novel therapeutic approach in promoting enamel health and mitigating the detrimental effects of radiation-induced caries. The synergistic action of laser therapy and fluoride application offers a promising avenue for managing dental complications in individuals undergoing head-and-neck radiotherapy, potentially improving their oral health outcomes and overall quality of life. While further research is needed to optimize treatment protocols and assess long-term efficacy, our findings contribute valuable insights to the development of effective preventive strategies for radiation-induced dental lesions.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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