

ORIGINAL RESEARCH

Evaluation of Primary and Permanent tooth Enamel Surface Morphology and Microhardness after Nd: YAG Laser Irradiation and APF Gel Treatment - An *In Vitro* Study

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ABSTRACT

Aims and Objectives: This study aims to evaluate and compare the morphological changes and hardness variations of enamel in control, lased, and combination of fluoridated and lased tooth surface.

Materials and Methods: Sixty primary teeth and 60 permanent teeth were sectioned into two equal halves and assigned randomly into three groups: Control group, Nd: YAG laser only, and 1.23% APF gel followed Nd: YAG lasing (laser-activated fluoride [LAF]). One half of each sample was kept in demineralization solution, and they were evaluated using microhardness (Vickers) tester and scanning electron microscope.

Results: Statistical analysis was performed. There was a significant increase in microhardness of enamel for LAF group and only laser groups after demineralization. In the laser alone group showed fine surface cracks and porosities before demineralization, and there was increased in cracks and craters after demineralization. In the LAF group, an irregular contour with marked crack propagation and glazed coalescence of fluorhydroxyapatite crystals before demineralization and increased homogenous surface coatings of fluoride covering the enamel after demineralization.

Conclusion: Nd: YAG laser irradiation and combined APF gel and Nd: YAG laser treatment of the primary and permanent tooth gave morphologically hardened enamel surface after demineralization.

Keywords: APF gel, Microhardness, Nd: YAG laser, Permanent tooth enamel, Primary tooth enamel, Scanning electron microscope.

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INTRODUCTION

Primary dentition is at a much higher risk for caries development than the permanent dentition, and the composition of primary enamel is considerably different with a higher organic content and a lower mineral content.^[1]

The past decades have seen dramatic changes in the field of dentistry, especially in preventive dentistry, with the discovery of fluoride and its role played in caries prevention which has not only strengthened but also has changed the perspective of dentistry to common man as well as professionals.^[2]

Most recently, the role of lasers in caries prevention has been explored. Initially, ruby laser was used to remove carious enamel and dentin. Subsequently, lasers of various types, i.e., argon, carbon dioxide, Nd: YAG (Neodymium-Yttrium-Aluminum-Garnet), Er: YAG (Erbium-Yttrium-Garnet), and diode laser producing either continuous radiation or pulsed beams were introduced.^[3-5]

Investigations related to the application of laser in the area of preventive dentistry have been carried out, indicating that human enamel irradiated with Nd: YAG laser was more resistant to acid decalcification than the unlasered enamel.^[6,7]

Another field of interest has been the exploration of combined use of laser and fluoride of developing new and more effective procedures for caries prevention. However, till date, detailed studies have not been reported about the use of Nd: YAG laser on enamel of primary tooth smooth surface along with fluoride.^[8]

The purpose of this study is to compare and characterize the surface morphological alterations and microhardness variations in primary and permanent teeth enamel after *in vitro* Nd: YAG laser irradiation alone and combination of Nd: YAG laser with APF gel treatment before and after demineralization.

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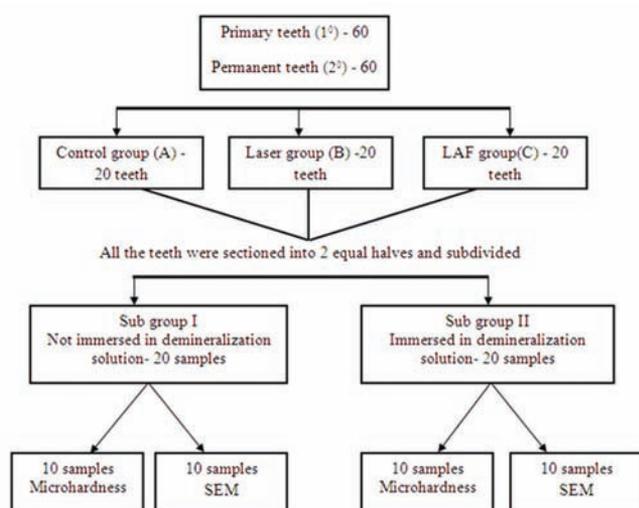
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MATERIALS AND METHODS

This study was conducted in the Department of Pedodontics and Preventive Dentistry, Mamata Dental College, Khammam. The teeth used for the study comprised non-carious extracted 60 retained/exfoliated primary teeth and 60 extracted permanent teeth. Then, all these samples were kept in individual sterile containers with storage medium of deionized water until they were mounted on dental stone blocks and subjected to APF gel treatment and laser application. Each tooth was sectioned into two equal halves.

Study Flow Chart showing control and experimental groups



Laser Procedure

Two halves of each tooth from group B in both primary and permanent teeth [Figure 2] were placed on the dental block made and irradiated with Nd: YAG laser of 1064 nm wavelength, 200 mJ energy, voltage of 550 V, 6 HZ frequency in pulsed mode for duration of 15 s according to manufacturer's instructions. Once the lasing was completed the enamel surface was cleansed with deionized water [Figure 1].

Laser-activated Fluoride (LAF) Procedure

In Group C, two halves of each tooth from both primary and permanent teeth were subjected to 4 min 1.23% APF gel treatment [Figure 3] according to the manufacturer's instructions, excess fluoride was removed and left it for 30 min for absorption of unbound fluoride along with bounded fluoride. Then, it was irradiated with Nd: YAG laser followed by thorough rinsing with deionized water for removal of excess fluoride.

Demineralization Procedure

One half of each tooth in Groups A, B, and C of both primary and permanent teeth were immersed in demineralization solution that is prepared with composition of 0.05 M acetic acid, 2.2 mM calcium, and 2.2 mM phosphate ions with pH - 4.3 for 24 h and washed with deionized water.

Standardized blocks of dental stone were made for both halves in each sample that has to be given for microhardness analysis [Figure 4] were mounted on it and kept in labeled containers, and other samples for scanning electron microscope (SEM) analysis [Figure 5] were also kept in labeled container. Analysis was done using SPSS version 14.

RESULTS

The primary tooth samples showed an interesting pattern of increase in microhardness Vickers hardness number (VHN) in comparison to control and treated groups. In Group B, there was significantly higher mean after demineralization ($389.32 \text{ VHN} \pm 23.40$ and $353 \text{ VHN} \pm 52.61$) than before demineralization ($360.30 \text{ VHN} \pm 39.07$ and $320.75 \text{ VHN} \pm 74.91$) in both primary and permanent dentition. In Group C, there was significantly higher mean after demineralization ($396.81 \text{ VHN} \pm 37.27$ and $360.35 \text{ VHN} \pm 43.89$) than before demineralization ($362.35 \text{ VHN} \pm 29.52$ and $336.65 \text{ VHN} \pm 45.25$) in both primary and permanent dentition.

Table 1 and Graph 1 show a marked increase in the microhardness (VHN) from control to the treatment groups. Table 2 and Graph 2 show intergroup comparison of mean values of microhardness before and after demineralization in all groups for primary and permanent teeth.

SEM Analysis in Primary Teeth

These micrographs of sound enamel surfaces from Group A [Figure 6] were relatively smooth with frequent enamel prism ends present on their surfaces. There are no areas of cavitations or surface defects. The surface showed shallow depressions and fine porosities within these depressions.

In Group B [Figure 7], micrographs of the primary tooth enamel possessed (a) irregular roughened surfaces with occasional areas of fine surface cracking and discontinuities. The surfaces were peppered with cavitations and craters, leading to an irregular undulated surface before demineralization. (b) These craters and cracks and small isolated porosities also increased after demineralization.

Table 1: Intragroup comparison of microhardness in primary and permanent teeth

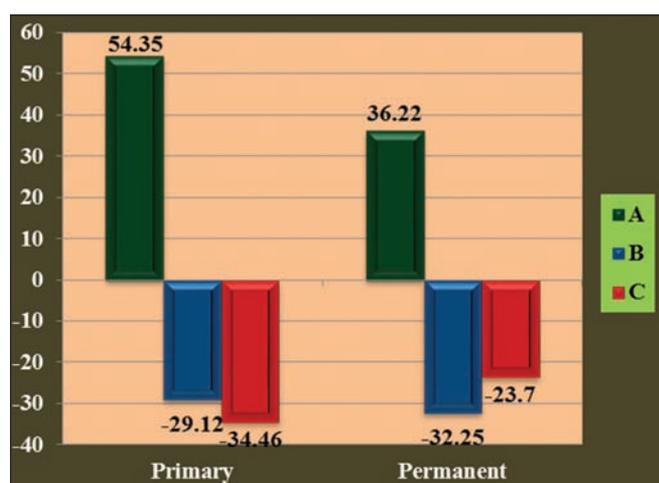
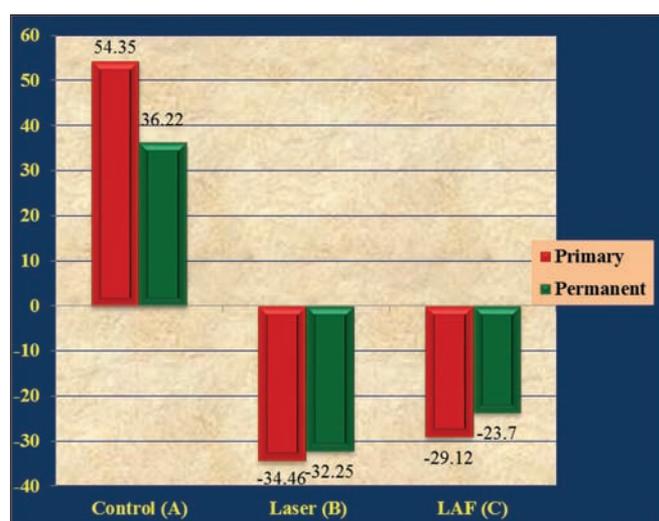
Teeth	Group	Before demineralization	After demineralization	P
		Mean±SD	Mean±SD	
Primary (1 ⁰)	Control (A)	361.86±27.82	307.51±57.48	0.007
	Laser (B)	360.20±39.07	389.32±23.40	0.006
	LAF (C)	362.35±29.52	396.81±37.27	0.004
Permanent (2 ⁰)	Control (A)	328.74±35.42	292.52±52.14	0.005
	Laser (B)	320.75±74.91	353.00±52.61	0.019
	LAF (C)	336.65±45.25	360.35±43.89	<0.001

*ANOVA analysis - $P < 0.05$ (significant). LAF: Laser-activated fluoride

Table 2: Intergroup comparison of mean values of microhardness before and after demineralization

Teeth	Groups			P	Post-hoc tests
	Control (a)	Laser (b)	LAF (c)		
	Mean±SD	Mean±SD	Mean±SD		
Primary (1 ⁰)	54.35±49.74	-29.12±25.79	-34.46±28.13	<0.001	Control>laser, Fluoride+Laser
Permanent (2 ⁰)	36.22±31.25	-32.25±35.67	-23.70±13.57	<0.001	Control>laser, Fluoride+Laser

*Post-hoc Turkey's test - $P < 0.05$ (significant). LAF: Laser-activated fluoride

**Graph 1:** Intragroup comparison of mean values of microhardness before and after demineralization**Graph 2:** Difference in mean microhardness values in all groups in primary and permanent dentition

In Group C [Figure 8], micrographs (a) surface coatings had irregular contours with numerous granular to

globular irregularities protruding from the surface coating and were quite porous with prominent fracturing pattern. Numerous cracked surface coatings covered almost the entire surface before demineralization and after that there was increase in glazed surfaces in different shapes (b).

SEM Analysis for Permanent Teeth

These micrographs of sound enamel surfaces from Group A [Figure 9] were relatively smooth with frequent enamel prism ends present on their surfaces. (a) There are no areas of cavitations or surface destructions seen. The surface showed shallow depressions and fine porosities within these depressions. The enamel surfaces are devoid of any surface deposits (b) smooth surface morphology with occasional depressions of 3–5 μm in diameters representing the prism end markings.

In Group B [Figure 10], micrographs of the primary tooth enamel possessed irregular roughened surfaces with occasional areas of (a) melting, recrystallization, fine surface cracking, and discontinuities. The surfaces were peppered with cavitations and craters, leading to an irregular undulated surface before demineralization. These (b) craters, cracks, and small isolated porosities were more glazed after demineralization.

In Group C [Figure 11], micrographs show the surface topography possessed what appeared to be surface coatings that masked the underlying enamel surfaces. These (a) surface coatings had irregular contours with more numerous granular to globular irregularities protruding from the surface coatings which are fluorhydroxyapatite crystals. Numerous cracked surface coatings covered almost the entire surface before demineralization and after there was increased in glazed surfaces in different irregular shapes (b) and had more prominent fracturing

patterns with fine cracks were seen. These patterns are more in permanent teeth than in primary teeth.

DISCUSSION

Laser irradiation of dental enamel has attracted a great deal of attention as it increases the resistance of dental

enamel to acid decalcification which is becoming popular in pediatric dentistry.^[9] Laser has its anticaries effect on sound enamel surface by changing its surface structure and physical properties including melting and recrystallization of hydroxyapatite crystals by improving its acid resistance further inhibiting surface demineralization.^[10]

In the present study, Nd: YAG laser with short



Figure 1: Sectioned tooth immersed in Deionized water

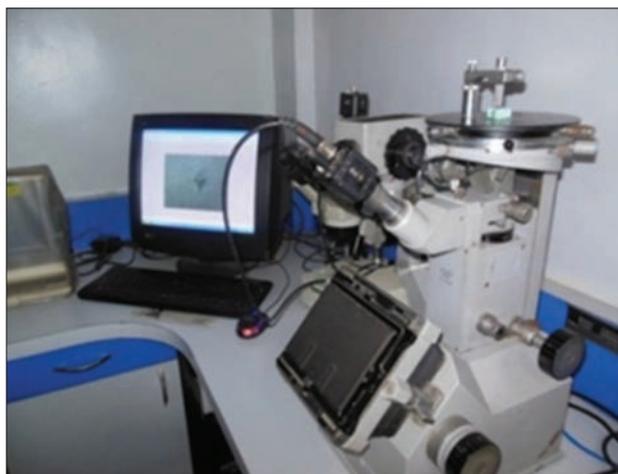


Figure 4: Vickers hardness tester



Figure 2: Application of Nd:YAG laser



Figure 5: Scanning Electron Microscope

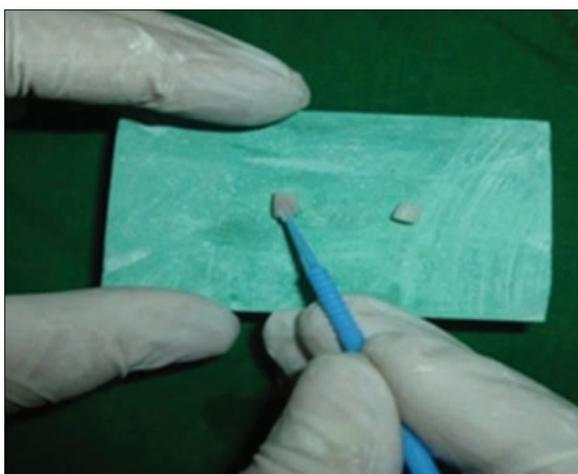


Figure 3: Application of APF gel



Figure 6: Group A(Control) in primary teeth

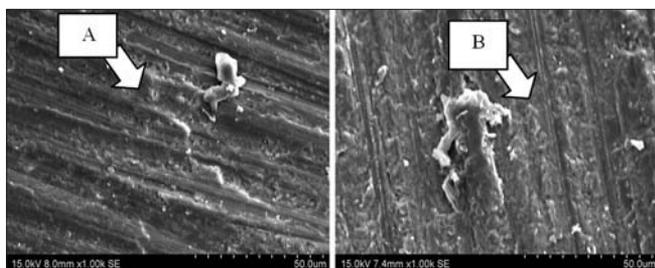


Figure 7: Group B (Laser) in primary teeth

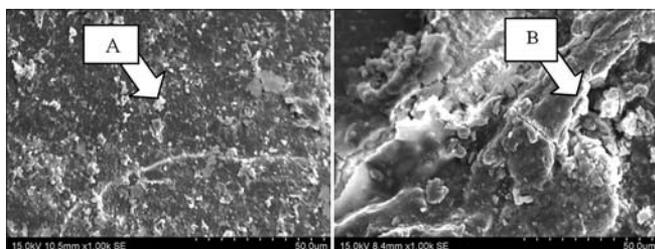


Figure 8: Group C (LAF) in primary teeth

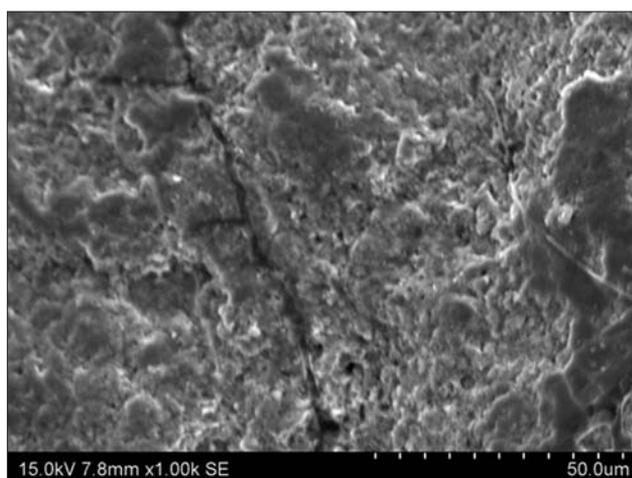


Figure 9: Group A (Control) in permanent teeth

pulsed wavelength is used to reduce thermal damage to adjacent tissue. It also has most accurate penetration into the targeted tissue by strong absorption.^[11,12]

Fluoride remineralizes incipient carious enamel and acts as chemotherapeutic agent by three mechanisms,^[13-15] (1) improving acid resistance of the enamel, (2) enhancing remineralization of the incipient lesion forming a fluorapatite-like low solubility veneer on the remineralized crystals, and (3) interfering with microorganisms by inhibiting bacterial metabolism and enzymatic process.

The present study LAF therapy showed increased acid resistance than the laser application alone in both primary and permanent teeth.^[6,11,16]

Microhardness was evaluated using Vickers hardness tester after demineralization of tooth samples showed that there was decrease in microhardness in control group with an increase in microhardness in laser group and maximum increase in LAF group.^[6,17,18] However, in contrary, some studies have shown

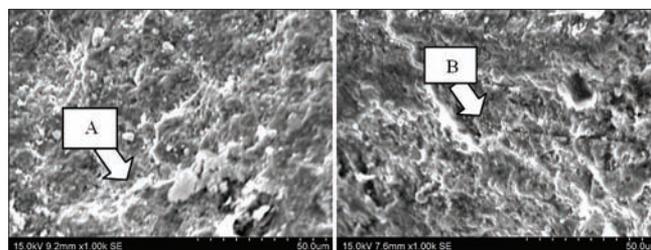


Figure 10: Group B (Laser) in permanent teeth

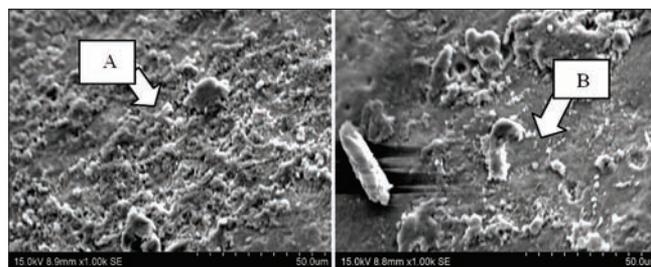


Figure 11: Group C (LAF) in permanent teeth

decrease in the microhardness values when enamel exposed to Nd: YAG laser after APF treatment.^[10,19]

In the present study, morphological alterations present in micrographs of laser group were showing minute cracks and crazing's with occasional craters and coalescence of hydroxyapatite crystals.^[20] In fluoride and laser group, surface enamel showed irregular contours with numerous granular to globular patterns due to coalescence of fluorhydroxyapatite crystals protruding from the surface coatings.^[1] This group after demineralization showed more areas of coalescence of globules and glazed surfaces.^[6,11,17-18]

CONCLUSION

Nd: YAG laser irradiation and combined APF gel and Nd: YAG laser treatment of the primary and permanent tooth enamel gave morphologically hardened enamel surface after demineralization. The topical fluoridation and laser combination seems to be a promising preventive measure for primary and permanent teeth. Further, clinical studies are needed to evaluate the acid resistance and in-depth knowledge regarding the preventive potential of lasers.

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