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Comparative Evaluation Of Microhardness And Flouride Release In Primary molar Enamel Treated With Acidulated Phosphate Flouride Gel Alone And In Combination With Diode Laser (810 nm) – An In Vitro Study

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Abstract

In spite of the various caries prevention methods available, prevalence of Early Childhood Caries remains high globally. Hence advanced methods are sought for, and lasers are proving their versatility in this aspect as well. Synergism in action of lasers and fluorides in caries prevention has been a topic of interest. This in vitro study intended to gather evidence regarding the action of diode lasers and Acidulated Phosphate Fluoride gel in various sequences of combination, on primary enamel. Primary molar enamel specimens were treated with Acidulated Phosphate Fluoride gel alone and in combination with diode laser in different sequences, which were then pH cycled. Laser parameters were set as 810 nm wavelength, power 1W, exposure time of 60 seconds and 600 μm diameter fibre was used for power transmission in non contact mode. Fluoride released in acidic medium (used in pH cycle) and enamel microhardness were measured. The obtained values were consolidated, ANOVA table was constructed and Tukeys analysis performed. There was a statistically significant difference in the microhardness and fluoride release between most of the groups. The group which was ‘lased followed by APF applied’ showed improved microhardness and fluoride release followed by the ‘simultaneous application group’ and ‘APF alone’ group in decreasing order. Diode laser can hence be added as a potential adjunct to topical fluoride in caries prevention of primary dentition. Lasing prior to Acidulated Phosphate Fluoride application was found to give better results than simultaneous application in this study. Laser activated fluoride therapy can therefore be added as a promising method to the current repertoire of ECC prevention strategies.

Keywords: Primary dentition, enamel, caries prevention, microhardness, fluoride release, Acidulated Phosphate Fluoride, diode laser, Laser activated fluoride therapy

Introduction

Although the prevalence of Early Childhood Caries has decreased over years, it remains to be a global health challenge despite the improvements in dental health services rendered across the world. National Oral Health Survey (1) conducted in India by the Dental Council of India in 2002–2003 shows that the prevalence of dental caries in children aged 5 years to be 50%. Prevention of ECC is of significance considering their consequences on the general health and social life of a growing child as well as the economic burden imposed on family and government.

Caries prevention modalities include reduction of dietary refined carbohydrates, use of improved plaque removal and oral hygiene techniques. In addition; use of systemic and topical fluoride applications, placement of pit and fissure sealants, use of fluoride releasing restorative materials, use of mouth rinses containing antimicrobials such as chlorhexidine gluconate, xylitol are all important.

As an adjunct to fluoride applications, previous investigations have demonstrated the potential of laser pre-treatment of enamel to inhibit subsequent acid-induced dissolution of enamel. Since the early research by Stern and Sognnaes, numerous studies have examined the combination of laser irradiation and topical fluoride application in order to verify if the laser energy could increase the resistance of dental structure to acid. (2) The technique of caries prevention provided through the combination of laser irradiation with topical fluoride application is termed as laser-activated fluoride therapy (LAFT). (3)Lasers were found to improve caries resistance of primary teeth enamel as well. (4-5) Diode lasers are very popular among practitioners owing to their low cost, being portable and not heavy weight, easy handling and application and can be used for LAFT. APF has numerous advantages making it an accepted formulation like ease of application and availability in numerous flavours which is advantageous for use in even a younger child. Hence APF and diode laser were selected in the study on LAFT in primary enamel.

The study compared the ‘microhardness’ and ‘release of fluoride ions in acidic medium’ from primary enamel specimens with APF gel applied alone and with diode penetration done before and through APF gel.

Materials And Methods

Teeth selection and grouping: Twenty freshly extracted or exfoliated sound deciduous maxillary/mandibular molars stored in distilled water were used in the study. (6) The root portion, if present, was sectioned approximately 1.0 mm below the cementoenamel junction and the crown of each tooth was bisected in a mesiodistal direction providing two blocks (one buccal and one lingual). The resulting 40 blocks were ultrasonically cleaned, a piece of adhesive tape was attached on the flattest portion of the buccal and palatal surfaces (after polishing with 300, 600 and 1200 grit silicon carbide papers to achieve a flat enamel surface)and the specimens were coated with two layers of enamel varnish. After complete drying of the coating, the tape was removed, leaving a window of approximately 4 mm^2 (2 mm x2 mm) of exposed enamel. The specimens were randomly assigned to 4 groups (n= 10) without the investigator being aware of the group and subjected to treatment of enamel surface according to the following group distribution:

Group 1 -control group (no treatment)

Group 2 - APF treatment group

Group 3- Application of 1.23% APF gel on the exposed enamel area, followed by irradiation with diode laser for 1 minute and removal of the gel after 4 minutes of contact with tooth surface.(APF+LASER group)

Group 4 - Laser application on the exposed enamel area for 1 minute, followed by application of APF gel for 4 minutes. (LASER-APF group)

Fluoride treatment: Application of 1.23% acidulated phosphate fluoride gel on the exposed enamel area for 4 minutes and thereafter to be removed with gauze.

Laser treatment: Laser parameters were tip diameter 600µm, in non contact mode, power 1W, and spot size of 4mm^2 with 1 minute irradiation time.

Ph cycling process: The pH cycling mode described by Featherstone et al and modified by Serra and Cury was followed in this study. (7-8) The specimens were individually immersed in 7 mL of a demineralizing solution (DES) (2 mmol/L of calcium, 2mmol/L phosphate and 75mmol/L acetate, pH 4.6) for 6 hours, followed by rinsing with distilled and deionized water for 10 seconds and gentle drying with absorbent paper. Then, they were individually immersed in the same quantity of remineralizing solution(RES) (1.5 mmol/L calcium, 0.9 mmol/L phosphate, 150 mmol potassium chloride and 20 mmol/L cacodilate buffer, pH 7.0) for 18 hours. All procedures were carried out at 37^{o}C. The DES and RES were renewed daily and the cycles repeated for 10 days. After every 5 days, the specimens were immersed individually in the RES for 2 days. The experiment lasted for a total of 14 days. The study was done after obtaining ethical clearance from Instituitional Ethical Committee.

Measurement of variables and statistical analysis: Amount of fluoride release in acidic medium was quantitatively estimated using ISE (ion specific electrode) technique. Surface microhardness was estimated using CLEMEX, JS 2000 micro hardness tester. The obtained values were imported into SPSS 14 software for statistical analysis. An ANOVA model was constructed (P value of 0.05), followed by Tukey’s test for multiple pair wise comparisons of mean values.

Results and Discussion

When control group was compared with other groups, both variables showed a statistically significant increase in treated groups; except for microhardness in APF and APF+Laser group which showed a decrease.

Pair wise comparison gave the following results

APF vs APF + Laser

The fluoride released when compared showed that values of APF + Laser group were more than that in APF group. This difference was found to be statistically significant.

The mean values of microhardness in the two groups when compared showed that ’APF < APF + Laser’. But the statistical analysis got a *p value greater that 0.05* which made the result statistically insignificant.

APF vs Laser – APF

The fluoride released when compared between the two groups was found as Laser – APF group better than APF group. This variation was found to be statistically significant.

The increased microhardness values of Laser – APF group when compared to APF alone group was established to be statistically significant. So, the result can be represented as ‘Laser – APF > APF’.

PF + Laser vs Laser – APF

The mean values of fluoride release in Laser – APF were more than that in APF + Laser group. This result also found to be statistically significant.

The mean values of microhardness in Laser – APF group was greater than that of APF + Laser group. The variation was shown to be statistically significant.

ECC affects the primary dentition which has a significant role to play in the growth and development of a child – be it anatomic, physiologic or psychologic. The caries preventive action of fluoride includes increased resistance to demineralization of the tooth surface, enhancing remineralisation of the demineralised tooth structure, inhibition of bacterial enzymes necessary for acid formation, inhibition of adhesion of plaque to the tooth structure and favourable modification of the size and shape of the tooth structure for caries resistance during the development of tooth buds. (9)Topical treatments with high fluoride concentration as in APF result in the formation of loosely bound fluoride (CaF_2) which acts as a potential reservoir of fluoride, enhancing remineralization and retarding demineralization processes. (10)

Laser is an inseparable part of modern dentistry. The action of lasers in caries prevention has been investigated by numerous authors since pioneer research by Stern and Sognnaes. (2) Caries inhibiting action of laser is proposed to be due to laser-induced compositional changes in enamel resulting in purer hydroxyapatite crystalline structure, the photo thermal effect causing reduction in enamel permeability, reduced enamel diffusion due to reduction in enamel diffusion, fusion and melting followed by resoldification of crystals, alteration of the organic matter, compositional differences (decrease in the amount of protein, carbonate and hydroxide), oxide formation and formation of high temperature phases (nonhydroxyapatite phases, apparently similar to tri- and tetracalcium phosphates). (11-15)

There are numerous studies showing the effectiveness of LAFT on permanent enamel, but those on primary enamel are less. The laser action on primary and permanent enamel can be distinct, due to the differences between these substrates. The mineralization, thickness, calcium, and phosphorus percentage is lower in enamel of primary teeth than in permanent teeth. (15) Despite immense researches, there is no consensus to date on whether fluoride should be applied before or after laser irradiation.

Microhardness

Microhardness measurements were utilized to assess the extent of protection afforded by LAF therapy. LAFT was found to be increasing the microhardness of permanent enamel by many investigators (16-21)

The mean microhardness values obtained in the different groups when compared, gave the following result.

Laser - APF > control > APF + Laser > APF

Microhardness was found to have reduced in two treatment groups in comparison to control group. This may be attributed to the adverse effect of the acidic preparation of fluoride gel used on microhardness values of thin enamel specimen in the current study. (3) (20) (22) The effect of LAFT on enamel was hence found to be improved by lasing the enamel prior to F application.

Study by Naveen Reddy et al using NdYAG and APF gel on primary enamel showed a statistically significant increase in microhardness of LAFT group over laser alone and control group. (23) In another study NaF and diode laser was combined on primary teeth and it was seen that though microhardness increased in LAFT group, there was no statistically significant increase over F alone group. An in vitro study conducted by M. R. L. A. Santaella, A. Braun, E. Matson, M. Frentzen however reported only an equivalent effect of fluoride varnish alone in comparison to its use in combination with diode laser. (24)

1.1. Fluoride release

Fluoride released into the acidic medium ie, the initial demineralising solution during pH cycling was assessed in the present study as a means to estimate the amount of loosely bound fluoride formed after LAFT.

The results gave the following outcome.

Laser – APF > APF + Laser > APF > control

The fluoride released measured also substantiated the improved properties of diode laser application prior to AFP gel.

In a study conducted by Patrick R. Schmidlina et al, estimated fluoride uptake was more when CO_2 laser was applied through topically applied amine fluoride. (19) In a study by M.C. Vitale et al, LAF treated enamel had more F content when compared to fluoride alone applied enamel. (25) In another study it was seen that increased formation and retention of CaF_2 like material was seen in LAFT enamel when compared to fluoridated ones. (26) It was seen in the study conducted by Y.R. Jeng etal that laser treatment increased F uptake by about 23%. (27) The above mentioned studies were done on permanent enamel.

The diode as well as CO_2 laser after NaF application on primary enamel showed an improved F uptake than F alone group in a study by Zahra Bahroloomi. (28)

This study was conducted simulating a few intraoral conditions under in vitro model to demonstrate the caries preventive action of LAFT. The fact that it was conducted in vitro is an inherent limitation to this study. The conclusions drawn in the present in vitro study need to be substantiated with in situ studies. Certainly additional research is warranted to propose a more specific mechanism of action which can be explained with chemical and ultra-structural minutiae involved in LAFT.

Table 1: pairwise *t test* summary

GROUPS	Microhardness (KHN)		Fluoride release(PPM)		Pair compared	t test result	
						Microhardness (KHN)	Fluoride release(ppm)
	MEAN	SD	MEAN	SD	1 vs 2	Pvalue 0.0001<0.05 (Significant)	Pvalue 0.000<0.05 (Significant)
Control (1)	325.4	17	1.4350	0.2165	1 vs 3	Pvalue 0.0060<0.05 (Significant)	Pvalue 0.000<0.05 (Significant)
APF(2)	285.7	17.08	2.250	0.292	1 vs 4	Pvalue 0.0007<0.05 (Significant)	Pvalue 0.000<0.05 (Significant)
APF	296.1	24.39	2.56	0.156	2 vs 3	Pvalue	Pvalue

+Laser (3)						0.1978>0.05 (Not Significant)	0.00041<0.05 (Significant)
Laser – APF (4)	349	6.63	2.915	0.193	3 vs 4	Pvalue 0.000<0.05 (Significant)	Pvalue 0.0013<0.05 (Significant)
					2 vs 4	Pvalue 0.000<0.05 (Significant)	Pvalue 0.000<0.05 (Significant)

Figure 1: Specimens of primary molar enamel

Figure 2 Application of APF gel

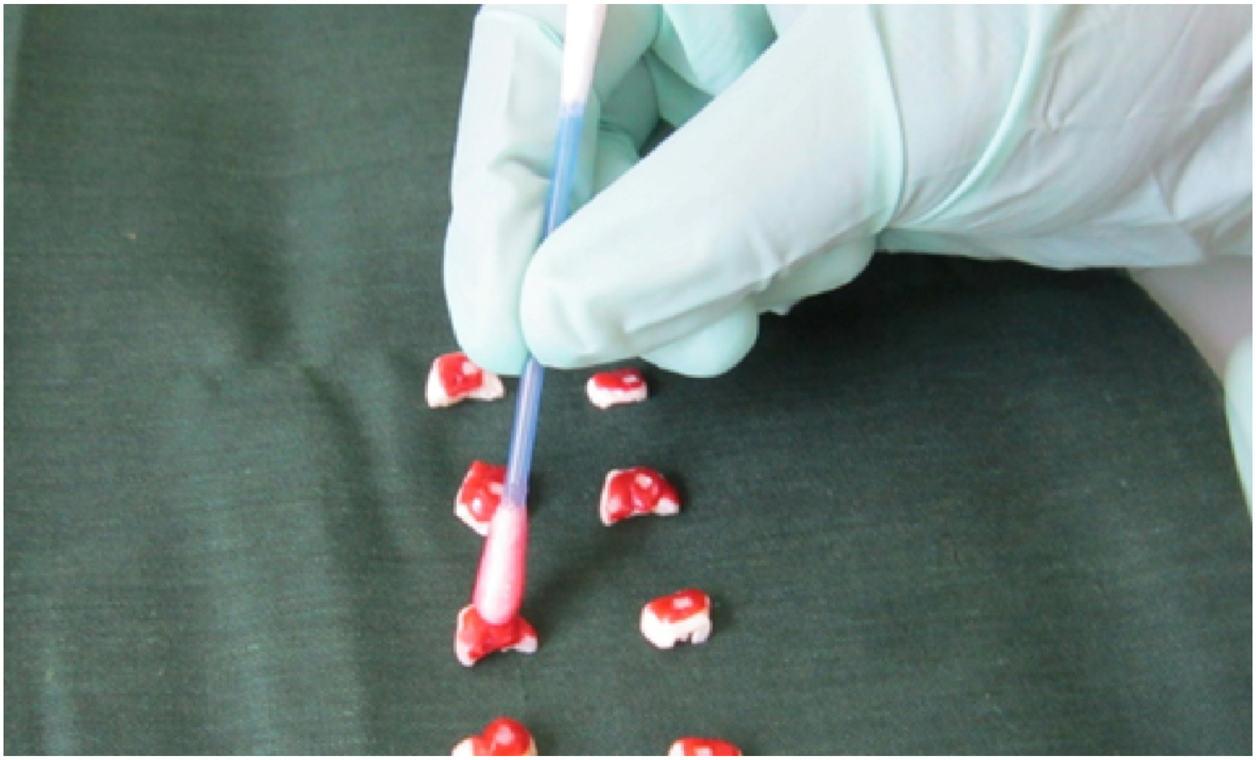

Figure 3: Lasing the specimens

Conclusion

Within the limitations of the study, diode laser was found to have synergestic action with APF in preventing caries of enamel in primary molars. The laser activated fluoride (LAF) treated specimens showed better microhardness and fluoride release when compared to APF alone treated ones. Of the two laser irradiation sequences used, the enamel specimens irradiated prior to fluoride application showed better properties when compared to ones which received simultaneous irradiation.

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