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THE EFFECT OF COMBINED TREATMENT BY LASER AND FLUORIDE ON THE ACID RESISTANCE OF ENAMEL IN PRIMARY TEETH (IN-VITRO STUDY)

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ABSTRACT:

INTRODUCTION: Er: YAG laser is contemplated as a preventive dental caries strategy when combined with topical fluoride varnish. Information regarding their combined effect on the enamel of primary teeth is still not established.

OBJECTIVES: The present study aimed to evaluate the effect of combined treatment by Er: YAG laser irradiation prior to topical fluoride varnish application on the acid resistance of enamel in primary teeth compared to fluoride varnish by elemental analysis.

MATERIALS AND METHODS: Twenty caries-free exfoliated human primary molars were painted with nail varnish, leaving one window 4 x 4 mm on the buccal surface. The samples were assigned to 2 groups (10 each), according to the surface treatment: (G I) 5% sodium fluoride varnish (Enamel Pro® Varnish) only and (G II) Er: YAG laser irradiation followed by sodium fluoride varnish. The baseline value for each specimen was recorded before the test procedure. The enamel specimens underwent pH changes over 14 days. EDX was used to assess the mineral content of the specimens after pH challenge.

RESULTS: A significant increase occurred in the mean values of the Ca at%, P at%, and Ca/P ratio in the enamel in both groups compared to the baseline values ($p \leq 0.001$). No significant difference was found in the mean percent change ($p \geq 0.05$) of the mineral content between the study groups.

CONCLUSIONS: The combined treatment by laser and fluoride is as efficient as fluoride varnish alone for increasing the acid resistance of enamel in primary teeth.

KEYWORDS: Primary teeth, fluoride varnish, Er:YAG laser, caries prevention.

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INTRODUCTION

Prevention is the primary aim of dentistry rather than the treatment of caries. Although caries prevention in permanent teeth has been improving greatly, yet it remains inefficient for primary dentition (1). Since the course of caries in children is progressive, more intervention procedures are needed to overcome this problem.

Over the years fluoride therapy proved to be the most effective method of increasing enamel resistance to acid dissolution (1-4). Different amounts of fluoride (F) uptake have been reported with the application of different fluoride-containing products. The topically-applied fluoride products are systems which supply fluoride to the surfaces of the dentition, at high concentrations. There are several evidence-based reports according to the American Academy of Pediatric Dentistry that in-office topical fluoride treatments in 5% NaF varnish forms or 1.23 percent F gel concentrations are effective in children with high susceptibility to caries (5-8).

Lasers are introduced as a pain-free curative tool in pediatric dentistry. Despite the utilization of lasers for drilling of dental tissues, the use of hard tissue erbium lasers such as Er: YAG has been considered for preventing enamel demineralization (9-11). The increased acid resistance of dental enamel following irradiation with lasers is related to physical and chemical alterations caused by photothermal and photochemical effects. These changes affecting the enamel's solubility may vary according to the temperature achieved by the laser during irradiation (12). Enamel solubility is assumed to decrease due to denaturation and swelling of the organic matrix that leads to blocking the diffusion pathway within the enamel. Above 200°C, a loss of carbonate also occurs that could contribute to increased acid resistance (13,14). Micro-spaces formed as a consequence of loss of water, and organic substances might prevent demineralization by trapping the dissolved ions available in the saliva or provided by the preventive agents (15). Cecchini et al., (16) used 7 different parameter settings (laser energy densities) ranging between 60 mJ - 135 mJ of Er: YAG laser and noted that lower (sub-ablative) energies decreased the solubility of enamel.

In addition to the previously mentioned strategies, the spotlight has been focused on combining laser irradiation with topical fluoride and remineralizing solutions (17, 18). Once the enamel surface is irradiated with laser it causes the melting of the enamel crystals which then coalesce to form a resistant crystal called pyrophosphate crystal which is said to form a completely impermeable barrier. When this is accompanied by fluoride application, the hydroxyapatite crystals of enamel are replaced by fluorapatite crystals which is also a very resistant crystal but not as much as pyrophosphate crystal. Lasers can augment the effect of fluoride in the structural configuration of enamel both superficially by forming calcium fluoride (CaF_2) and also altering its crystalline structure (19,9). Bharti et al., (20) found a reduction in caries progression with the use of laser and topical fluoride combinations in primary teeth when compared to sodium fluoride varnish alone using Er,Cr:YSGG laser. On the other hand, Steiner-Oliveira et al., (19) observed no additional significant demineralization inhibitory effect using laser-fluoride combined treatments.

Even though the outcome of laser irradiation on the acid resistance of enamel is recognized in permanent teeth, nevertheless, the benefit of the combined therapy of Er: YAG laser and fluoride in primary teeth is still not certainly established.

Therefore, the study aimed to evaluate the effect of combined treatment by Er: YAG laser (Doctor Smile Pluser®, Italy) irradiation prior to topical fluoride varnish (Enamel Pro fluoride, Premier® Dental Products, USA) application on the acid resistance of enamel in primary teeth compared to fluoride varnish by Energy Dispersive Xray Spectrometer.

The null hypothesis of this investigation is that there will be no difference between the combined treatment by laser and fluoride, and fluoride varnish alone in the mineral changes of the enamel.

MATERIALS AND METHODS

Study design and setting

This was an experimental in-vitro study. It was performed in the Department of Pediatric Dentistry and Dental Public Health, Department of Oral Biology in Faculty of Dentistry, and the Department of Geology, Faculty of Sciences, Alexandria University.

Sample size

A total sample of 20 teeth was needed. It was estimated by adopting a power of 80% to detect a standardized effect size in the fluoride content of d=0.619 (21,22). The minimum sample size was calculated to be 10 teeth per group including a 10% increase to make up for processing error (23). The calculation of sample size was performed by GPower version 3.1.9.2. (24).

Study sample

Twenty recently exfoliated human primary molar teeth were collected from the outpatient dental clinic, Alexandria University hospitals. Molars were cleaned to remove blood and debris, then examined under a stereomicroscope and chosen free of any developmental anomalies, cracks, fissures or stains.

Ethical considerations

This study was approved by the Scientific Research Ethical Committee, Faculty of Dentistry, Alexandria University, Egypt.

Materials

Er: YAG laser (Doctor Smile Pluser®, Italy), 5% sodium fluoride varnish (Enamel Pro fluoride, Premier® Dental Products, USA) and the De/Remineralizing solution preparation (This solution was approximated to the supersaturation of dental minerals found in saliva) (25).

Method

Sample preparation

Twenty recently exfoliated, non-carious primary molars were collected and examined carefully. Nail varnish was painted on the buccal surface of every molar, thereby creating a single 4mm×4mm window in the middle third of the crown.

Grouping and enamel surface treatments

The specimens were randomly assigned to 2 groups (10 each) according to enamel surface treatment:

Group I (control): Each specimen was treated with a topical coating of Enamel Pro varnish (Premier® Dental Products Company, USA) (26).

Group II (experimental): Each specimen was manually irradiated by Er: YAG laser (Doctor Smile Pluser®, Italy) first, followed by a topical coating of Enamel Pro fluoride varnish.

The laser parameters used were: 40 mJ, 0.2W, 5 Hz with a beam diameter of 400 µm for 60 sec for every specimen (Doctor Smile Pluser®, Italy).

Method of varnish application: A thin, even film of the test varnish was applied using a one-use plastic brush on the tooth surface according to the manufacturer's instructions (26).

After the treatments, specimens were then stored at 37°C in distilled water for 24-hours, then they were all subjected to a cariogenic acid challenge.

pH cycling Model

All specimens underwent a pH cycle in 5ml of alternating solutions for 14 days. Each cycle consisted of 6 hours of demineralization and 18 hours of remineralization daily (27). Between each cycle, specimens were flushed with distilled water. The demineralizing solution consisted of; 2.2 mM calcium chloride ($CaCl_2$), 2.2 mM potassium dihydrogen phosphate (KH_2PO_4), 0.05M acetic acid (CH_3COOH), 1 M potassium hydroxide (KOH) that was used to adjust the pH to 4.4. On the other hand, the remineralizing solution (pH=7) comprised of 1.5 mM calcium chloride ($CaCl_2$), 0.9 mM sodium dihydrogen phosphate (NaH_2PO_4), 0.15 M potassium chloride (KCL) (25).

Energy dispersive x-ray spectrometer (EDX) evaluation

After the pH cycle, specimens were flushed with distilled water and prepared for evaluation quantitatively using EDX. Each specimen was mounted on a copper stub and analyzed using EDX (Jeol JSM-5300 Scanning Electron microscope). Elemental content distribution of calcium (Ca) and phosphorus (P) elements weight % of enamel were attained in the form of peaks on a graph with their corresponding readings. The Ca and P content were converted into the Ca/P ratio for each group (28).

All specimens were analyzed using energy dispersive x-ray spectrometry (EDX) before any treatment to record baseline values and after the intervention.

Statistical analysis

IBM SPSS software package version 20.0 (Armonk, NY: IBM Corp) was used for data analysis. Verification of normality of the distribution of variables was done by the Kolmogorov- Smirnov test. The normally distributed quantitative variables were analyzed by the Student t-test to compare the two study groups. Whereas, the Paired t-test compared between the different periods. Mann "Whitney test" was performed to compare the groups for not-normally distributed quantitative variables. The implication of the obtained results was judged at $p \leq 0.05$.

RESULTS

Table 1 and figure 1 show that there was no significant difference in the Ca content mean values between the study groups before (p=0.877) and after (p=0.101) the intervention. There was a significant increase in the Ca content mean values after the intervention within the 2 groups with $p < 0.001$ in both groups. The mean and standard deviation increase in Ca was 4.30±2.63 in group II (test) higher than group I (control) (2.92±1.41) with no significant differences.

Table 2 and figure 2 show that the P content mean values did not differ significantly between the study groups before (p=0.101) and after (p=0.505) the intervention. Moreover, the P content increased significantly within the 2 groups after the intervention with $p < 0.001$ in both groups. Group II (LF) had a higher percent change in P content than group I without significant difference.

Table 3 and figure 3 show that comparisons of Ca/P ratio mean values between the study groups before and after the intervention indicated no differences with p values of 0.383 and 0.495, respectively. A significant increase in the mean values of the Ca/P ratio after the intervention within the 2 groups with $p < 0.001$ in both groups. Although the mean percent increase in the Ca/P ratio of group II was higher than in group I, no significant difference was found between the 2 groups. The mean % change in group II (LF) was higher than group I without significant difference.

Table (1): Calcium content before and after intervention among the study groups

	Group I fluoride (n = 10)	Group II (Laser + Fluoride) (n = 10)	Test of sig.	p
	Mean ± SD.	Mean ± SD.		
Ca				
Before	58.4 ± 0.28	58.42 ± 0.32	t=0.157	0.877
After	60.1 ± 0.67	60.93 ± 1.34	t=1.762	0.101
p_0	<0.001*	0.001*		
% of change	↑2.92±1.41	↑4.30±2.63	U=35.0	0.280

t: Student t-test U: Mann Whitney test

p: p-value for comparing between the studied groups

p_0: p-value for **Paired t-test** for comparing between **before** and **after**

*: Statistically significant at $p \leq 0.05$

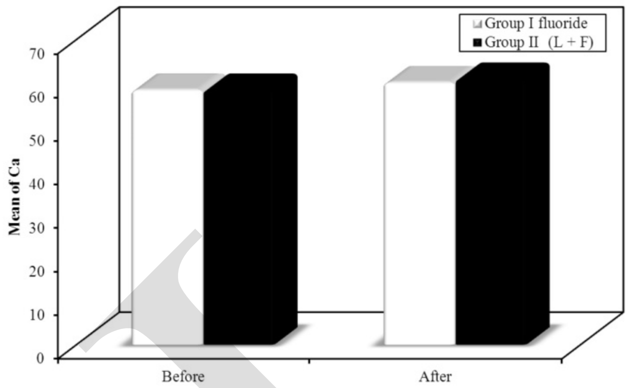

Figure (1): Calcium content before and after intervention among the study groups

Table (2): Phosphorous content before and after intervention among the study groups

	Group I fluoride (n = 10)	**Group II (Laser + Fluoride) (n = 10)**	**Test of sig.**	**P**
	Mean ± SD.	**Mean ± SD.**		
P				
Before	39.9 ± 0.67	39.07 ± 1.34	t=1.762	0.101
After	40.9±0.32	40.6±0.42	t=0.681	0.505
p_0	<0.001*	0.001*		
% of change	↑2.52±1.21	↑3.89±2.71	U= 35.0	0.280

t: Student t-test U: Mann Whitney test

p: p-value for comparing between the studied groups

p_0: p-value for Paired t-test for comparing between before and after

*: Statistically significant at $p \leq 0.05$

Figure (2): Phosphorous content before and after intervention among the study groups

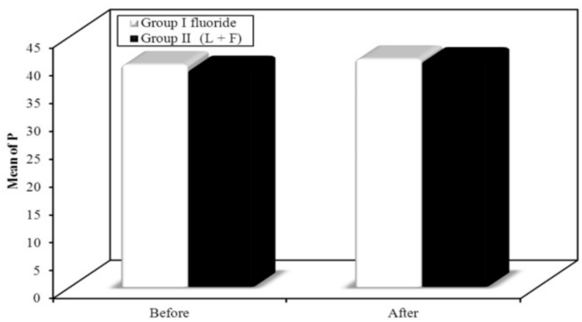

Table (3): Ca/P ratio before and after intervention among the study groups

	Group I fluoride (n = 10)	Group II (Laser + Fluoride) (n = 10)	Test of sig.	P
	Mean ± SD.	Mean ± SD.		
Ca/P ratio				
Before	1.46±0.02	1.50±0.05	t=0.894	0.383
After	1.47±0.03	1.50±0.05	t=0.696	0.495
p_0	0.001*	<0.001*		
% of change	↑0.38 ± 0.26	↑0.40±0.24	U= 39.0	0.436

t: Student t-test U: Mann Whitney test
p: p-value for comparing between the studied groups
p_0: p-value for Paired t-test for comparing between before and after
*: Statistically significant at $p \le 0.05$

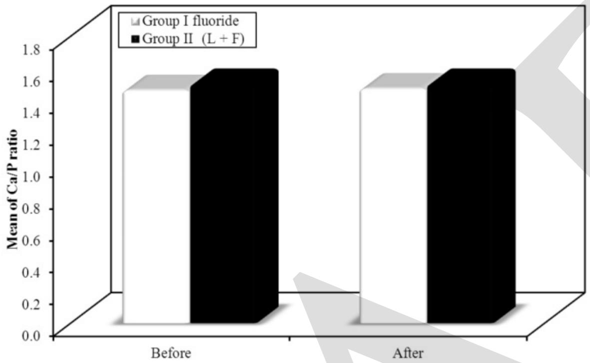

Figure (3): Ca/P ratio before and after intervention among the study groups

DISCUSSION

The findings of this in vitro study does not support the rejection of the null hypothesis that there is no difference in the mineral changes of enamel in primary teeth when treated by the combined application of laser and fluoride when compared to fluoride varnish alone.

In the present study, the 14-day pH cycling model used closely mimicked the pH fluctuations associated with the regular course of caries development to imitate the dynamic mineral loss and gain in the oral cavity in high caries risk individuals (27,29).

In the current study, the laser and fluoride combined application increased the enamel acid resistance as evident by a significant increase in Ca at%, P at% and Ca/P ratio after the intervention when evaluated by EDX (elemental analysis). The EDX quantitatively measures the levels of Calcium and Phosphorus in enamel both at baseline before any interventions and after the pH cycling, to detect the mineral changes that have occurred (30). This indicated that the combined use of laser and fluoride treatment is effective in preventing enamel demineralization.

The study results are consistent with Ceballos-Jiménez et al., (31) who stated that the combination of Er: YAG laser with NaF gel (1.1%) provoked a significant increase in mineral content and showed superior acid resistance to the demineralization of enamel compared to baseline values.

The Ca/P molar ratio is considered a genuine mineralization indicator that allows the establishment of behavior patterns, independent of variations of other elements in the teeth (32). Our findings showed chemical changes that intensify the mineral content of the enamel structure in terms of Ca/P ratio as a combined treatment group (laser+ fluoride) showed an increase in the mean percent change after intervention. Zamudio-Ortega et al., (32) had similar results and found that Ca and P at% as well as Ca/P ratio all have improved in enamel when treated at 12.7 J/cm2 and 39.8 J/cm2 with laser – fluoride combined protocols. Reformation of the organic matter through the reduction of carbonate content in enamel irradiated by Er: YAG could be a possible clarification for the study's discoveries (33). Moreover, the decrease of carbonate content renders the hydroxyapatite more insoluble, since the replacement of carbonate results in an unstable enamel crystal and, consequently, can increase solubility (34).

Concerning the control group (Enamel-Pro fluoride varnish group), a significant increase of Ca at%, P at% and Ca/P proportion resulted after treatment. Cochrane et al., (35) found that Enamel pro varnish releases calcium, fluoride and significant levels of inorganic phosphate making them readily available for uptake by tooth enamel. Our study results are following Ulkur et al., (36) who tested fluoride varnishes containing calcium phosphates and proved their efficiency against demineralization compared to Er: YAG laser irradiation alone.

When comparing the study groups, no significant difference resulted as regard Ca at%, P at% and Ca/P ratio between both groups after treatment. Although group II (combined treatment) showed a slightly higher mean percent elemental changes compared to the fluoride group however these changes were not significant. This discovery complies with Zotti et al. (37) who compared the combined treatment of Naf + laser to NaF alone and Er: YAG laser alone. They found that NaF + laser group resulted in the highest values of enamel microhardness when compared to the other 2 test groups with no significant difference between them. This could be due to crystallographic transformations that occurred on the surface of enamel which aids in the incorporation of fluoride and other minerals into the crystals increasing the elemental content measured by the EDX (37).

On the other hand, Ceballos-Jiménez et al., (38) compared sodium fluoride (NaF) Er: YAG laser irradiation (L), hydroxyapatite-NaF-xylitol, and combinations of laser with each agent and found a significant increase in the elements and the Ca/P ratio between the study groups with the maximum increase in laser + NaF group. Their results revealed that the application of 1.1% NaF gel on permanent teeth produced a significant increase in F at% in the dental structure leading to a stable Ca/P ratio. They indicated that Er: YAG laser single-handedly or in combined protocols is useful in creating a synergetic effect.

The possible limitations of the present study were the high cost of laser equipment and that the controlled environment of the study lacked some of the natural oral conditions. The pH cycling model didn't entirely simulate the complex oral conditions where the pH fluctuates more frequently along with external factors. The attained levels of pH changes depend upon the individual's dietary lifestyles, oral hygiene regimens, fluoride protocol and the constitution of human saliva as well as plaque nature for each individual.

CONCLUSIONS

Based on the limitations of this study it is concluded that:

The combined treatment by Er: YAG laser and Enamel Pro fluoride varnish, was as effective as Enamel Pro fluoride varnish alone for increasing acid resistance.

The use of both Enamel Pro fluoride varnish and Er: YAG laser proved effective in increasing the mineral content of enamel.

CONFLICT OF INTREST

The authors declare that they have no conflict of interest.

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