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The Effect of Combination of Er, Cr: YSGG Laser Irradiation and Clinpro White Varnish on Enamel Resistance to Caries: An in Vitro Study

[1]Hussein Sami Abdullah, B.D.S. and[2] Aisha Akram Qasim, B.D.S. ,M.Sc.
[1]B.D.S. ,M.Sc. student, Directorate of Health/Basrah, Basrah, Iraq
[2]Department of Preventive Dentistry, College of Dentistry, University of Mosul, Ninavah, Iraq
** Corresponding author at Department of Preventive Dentistry, College of Dentistry, University of Mosul, Mosul, Iraq*

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ABSTRACT
Background: This study aims to investigate the efficacy of Er,Cr:YSGG laser combined with Clinpro White varnish in preventing of enamel caries.
Materials and Methods: Freshly extracted thirty sound upper first premolars teeth were divided into three groups, each group of ten samples. Group (C): only washed with deionized water. Group (V/L1): First Clinpro White varnish was applied then Er,Cr:YSGG laser(0.25watt, 2.8 J/cm^2, 20 Hz, 11% air, 0% water). Group (L1/V): Er,Cr:YSGG laser (0.25watt, 2.8 J/cm^2, 20 Hz, 11% air, 0% water) was irradiated followed by Clinpro White varnish. Then, the pH cycling model was used to induce artificial caries. The effect was studied in vitro using Vickers microhardness test and the obtained results were statistically analyzed.
Results: There was reducing in surface microhardness in all groups due to the demineralization. Statistically there was no significant difference between VL1 and L1V groups whileboth of them had a highly significant resistance against microhardness loss compared with control group of deionized water.
Conclusions:The impacts on enamel resistance to demineralization and reducing its solubility seem to indicate that there is no difference in usingEr,Cr:YSGG laser at 0.25watt as a pretreatment and post treatment for Clinpro White varnish.
Keywords: Er,Cr:YSGG Laser, Enamel demineralization, Clinpro White varnish, Microhardness.

I. INTRODUCTION

Dental caries is one of the oldest and most common human diseases. It is a dominant infectious and prevalent disease caused by particular tooth-adherent bacteria, primarily streptococcus mutans that metabolize sugars and release acid, which over time demineralizes the structure of the tooth [1].

Demineralization can be reversed in its early stages through uptake of calcium, phosphate, and fluoride. Fluoride acts as a catalyst for the diffusion of calcium and phosphate into the tooth. The rebuilt crystalline surfaces, composed of fluoridated hydroxyapatite and fluorapatite, are much more resistant to acid attack than is the original structure [2].changes have been made on fluoride varnishes to include calcium and phosphate ions within varnish buildingin an effort to further improve its effectiveness [3].

Functionalized tri-calcium phosphate, or fTCP, is a "smart" calcium phosphate device that allows calcium and phosphate ions supply to the teeth and acts synergistically with the addition of fluoride to improve efficacy [4].

Although dental caries is a preventable disease, it is still common and remains a public health problem, especially in developing countries, and certain populations in economically developed countries [5]. Therefore, there is still a need to prevent dental caries and search for alternative methods for disease prevention. Erbium lasers (Er:YAG and Er, Cr:YSGG) being hard tissue lasers are developed more recently than other laser types, Erbium, chromium: yttrium-scandium-gallium-garnet (Er,Cr:YSGG) laser ,It has a wavelength of 2.79 µm [6], Erbium lasers have been proposed to prevent enamel and dentin demineralization by ''Laser-Induced Prevention of Demineralization'' (LIPD), a mechanism by which erbium laser irradiation causes thermal changes in enamel, resulting in chemical and/or morphological structure modifications without ablation [7].

Lasers have also been used in combination with fluoride for caries prevention and this technique is termed as laser-activated fluoride (LAF) therapy[8].The association of laserirradiation with fluoridated products can be a

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most promissory alternative to increase the enamel resistance to demineralization[9].

Some controversies still exist in the literature regarding the appropriate Er,Cr:YSGG laser parameters, when used in combination with fluoride component containing calcium and phosphate, and their efficacy according to the order of application in decreasing the solubility of enamel [8,10].

The significance of the study is to attainadditional prevention of enamel demineralization centering on Er,Cr:YSGGlaser irradiation combined with Clinpro White varnish which are hoped to give promising synergistic results.

The aim of the present in vitro study was to evaluate the effect of the Er,Cr:YSGG laser in combined withClinpro White varnish, on enamel resistance to demineralization using artificial demineralizationtaking in our consideration the order of treatment. The effect was studied in vitro using Vickers microhardness test.

II. MATERIALS AND METHODS

Ethics Statement:

This study protocol was performed in vitro, submitted and achieved by the Local Ethics Committee (UoM.Dent/ H.L.3/ 21) Research Ethics Committee of Collage of Dentistry, University of Mosul, Nineveh, Iraq.

Teeth samples preparation:

A randomized controlled in vitro study design was used.Freshly extracted thirty soundupper first premolars teeth with intact buccal enamel surface were collectedfrom individuals (16-25) years old who were received orthodontic treatment in private orthodontic clinics in Basra. The teeth were examined visually. Sound teeth (no visible evidence of a carious lesion) were involved in the study. The teeth with restorations, caries, discoloration, fractures, enamel developmental and malformations were excluded from the study. Theextractedteethwerecleaned,all remnants were removed, then rinsedwithdeionizedwaterandmaintainedinthymolsolution0.1%to avoid dehydration andinhibitthegrowth ofbacteria[11]. Following polishing the teeth with non-fluoridated pumice, the remaining roots were cut 2 mm below the cemento-enamel junctions with a straight diamond bur using sufficientwater irrigation to avoid harming the enamel, then the crown pulp was removed with an excavator. All samples were checked under a dental microscope for any cracks or enamel defectsand then inserted in chemical cured resin in plastic rings [6].The plastic rings were cut and constructed so that their upper and lower surfaces were parallel. Each ring included a single tooth that was fixed in the center of the ring's upper surface, exposing the tooth's buccal surface. The middle 1/3 of the crown's buccal surface was estimated using a caliper by measuring the distance between the cemento-enamel junction and the cusp tip, as well as the mesio-distal dimension. An adhesive tape of (4×2mm^2) was fixed on the middle 1/3 of the crowns. Then, the crowns were coated with acid-resistant varnish (Rimmel, London, UK) except for a (4×2mm^2) window of exposed enamel [7]. Then the samples were dried, and tapes were removed to expose the enamel.

Materials and equipments:

1. Clinpro White Varnish that contain 5% Sodium fluoride (2.26% or 22.600 ppm of the fluoride ion) and functionalized tricalcium phosphate (fTCP) exclusively from 3M ESPE, made in USA, expire date 28/7/2022.
2. Er,Cr:YSGG laser system (Waterlasei-Plus,Biolase Technologies Inc., San Clemente, CA USA) that emits photons with 2.78 μm wavelength With parameter of (0.25watt, 2.8 J/cm^2, 20 Hz, 11% air, 0% water) [12]. For the handpiece, the MZ6 tip was chosen at the focal area with a beam diameter of 600 μm in order to avoid overheating the pulp of the teeth [13]. To maintain a constant spot size during the irradiation process, an endodontic file with a rubber stop was attached to the fixed handpiece with the distance of 1 mm from the enamel surface [14].Also,water cooling is not always necessary [7]. The repetition rate was set to 20 Hz according to [6]. The handpiece was fixed, in its handle, perpendicular to the enamel surface. The specimens were irradiated once for 10 seconds through slowly rotating the specimens horizontally by the hand to ensure uniform irradiation and coverage of the entire exposed sample area [12].

Table (1) were clearly shown the irradiation parameters for laser category.

Table (1): Er,Cr:YSGG laser irradiation parameters used in the present study.

Laser group	Power (W)	Energy density (J/cm^2)	Repetition rate (Hz)	Irradiation time (s)	Water	Air

Er,Cr:YSGG	0.25	2.8	20	10	NO	11%

Experimental design of the study:

To facilitate identification, each tooth was assigned a unique number between 1 and 30. Each group of ten teeth was stored at room temperature in its own beaker labeled with the group name and containing 200 ml of deionized water solution. Following that, each tooth in each group was separately placed in 10 ml deionized water in a plastic jar labeled with the tooth group's name and number [15]and then exposed to the following caries prevention method:

- **Group (C) (control group):** no agent was applied, only washed with deionized water.
- **Group (V/L1):**First Clinpro White varnish was applied on the window of specimens' surface for 4 minutes according to the manufacturer's instructions. The specimens then were stored in deionized water. After 24 hours, the varnish layer was removed by rubber cap and low-speed handpiece. Then, Er,Cr:YSGG laser (0.25 watt, 2.8 J/cm^2, 20 Hz, 11% air, 0% water) was irradiated as explained previously on the window of specimens' surface in a scanning style [12].
- **Group (L1/V):**Er,Cr:YSGG laser (0.25 watt, 2.8 J/cm^2, 20 Hz, 11% air, 0% water) was irradiated as explained previously on the window of specimens' surface in a scanning style followed by application of Clinpro White varnish for 4 minutes according to the manufacturer's instructions. The specimens then were stored in deionized water for 4 minutes, and then the varnish layer was removed by rubber cap and low-speed handpiece[12].

Then, the pH cycling model was used to produce artificial caries in all treatment groups through a cycle of demineralization and remineralization. Also, all treatments were achieved by skillful operator and the evaluation was done blindly.

pH-Cycling:

Artificial caries was produced by exposing teeth samples to PH cycling. The teeth samples were immersed in a Demineralizing solution ($CaCl_2$ 2.2mM, NaH_2PO_4 2.2mM, and acetic acid 0.05M, PH of 4.5, adjusted with KOH 1M) for three hours and then in a Remineralizing solution ($CaCl_2$ 1.5mM, $NaHPO_4$ 0.9mM, and KCl 0.15mM, PH of 7.0) for twenty hours [16]. Teeth samples were briefly rinsed with deionized water between solutions and placed in artificial saliva composed of NaCl 0.40, KCl 0.40, $CaCL_2.2H_2O$ 0.79, $NaH_2PO_4.2H_2O$ 0.78, $NaS_9.H_2O$ 0.005, $CO(NH_2)_2$ Urea 0.1, in 1000 ml Distilled water, PH of 7 (concentration G \ L) for 30 minutes at the end of the demineralization process and 30 minutes at the end of the remineralization process [17]. The teeth were subjected to a total of ten cycles, each cycle was lasting for one day (24 hours). Every day, the demineralizing and remineralizing solutions were changed, and the artificial saliva was replaced after each treatment [16]. Preparation of the chemical solutions was done by the researcher at the Marine Science Centre in University of Basrah.

Microhardness assessment:

All specimens were tested for their microhardness on two occasions: first before the treatments (baseline), and then at the end of the pH cycling stage (post-treatment). A Vickers hardness tester (Jinan Kason Testing Equipment Co., Ltd., China) with the load of 500 gm for 15 seconds was used (according to the instruction of the machine) for assessing the microhardness. For all samples, the load and time were constant. Three indentations were made at the exposed labial enamel surface of each sample, and the average value was recorded as the microhardness of each specimen [18].This test was conducted at University of Basrah, Collage of Engineering, Department of Mechanic.

$VHN= (kg \backslash mm2) = 1.854 \times P \backslash d^2$

P= the testing load in grams.

d= the length of the diagonal line across the indentation in microns.

Statistical analysis:

Data were analyzed using the Statistical Package for Social Sciences software program (IBM SPSS Statistics 26). Shapiro–Wilks test was applied to find out the distribution type of experimental measurement data. As the data were normally distributed, the one-way ANOVA test and Duncan[a] test enabled us to decide whether there are any statistically significant differences between the groups or not. A p-value of less than 0.01 was considered statistically significant.

III. RESULTS

Indentation surface microhardness test results:

According to the obtained measurements of this study, table (2) showed the descriptive statistics including means, standard deviations, minimum and maximum values in addition to the numbers of the samples of tested groups before and after pH cycle. Based on the means values for tested groups after pH cycle, the groups of lasers thenvarnish andvarnish then laser had the least reduction in the surface microhardness mean value.

Table (2): Descriptive statistics of microhardness measurements among tested groups at baseline and after pH cycle.

Groups \ Variables		Baseline	After pH Cycle
Deionized water (C)	**Mean**	**310.4270**	**190.1150**
	Std. Deviation	**11.77794**	**7.80915**
	Min.	**295.12**	**175.32**
	Max.	**328.00**	**199.75**
	N	**10**	**10**
Varnish +Er,Cr:YSGG Laser (VL1)	**Mean**	**306.6640**	**255.8940**
	Std. Deviation	**11.52762**	**5.81813**
	Min.	**289.12**	**248.98**
	Max.	**330.00**	**267.76**
	N	**10**	**10**
Er,Cr:YSGGLaser + varnish (L1V)	**Mean**	**306.6800**	**260.3090**
	Std. Deviation	**11.31297**	**7.60311**
	Min.	**291.00**	**245.67**
	Max.	**329.70**	**270.32**
	N	**10**	**10**

Table (3) ANOVA test explains that there was no significant difference for the surface microhardness readings existed among the tested groups at baseline at p≤0.01, while after pH cycle, there was a high significant difference among tested groups at p≤ 0.01.

Table (3): ANOVA test between tested groups at baseline and after PH cycle respectively at p≤ 0.01.

Microhardness		Sumof Squares	Df	Mean Square	F	Sig.
Baseline	**Between Groups**	94.001	2	47.001	.353	.706
	Within Groups	3596.303	27	133.196		
	Total	3690.304	29			
AfterPH Cycle	**Between Groups**	30911.889	2	15455.945	303.771	.000
	Within Groups	1373.767	27	50.880		

	Total	32285.656	29			

***highly significant difference at p≤ 0.01**

Duncan[a] multiple analysis range test was done to further explain that there was a highly significant difference of microhardness values for groups after pH cycle existed at p≤ 0.01. All groups were arranged in nonhomogeneous subsets of data representing the surface microhardness means values of each group after pH cycle at which there were no significant difference between L1V group and VL1 group and both of them had a highly significant resistance against microhardness loss, while the least value of surface microhardness belonged to the control group of deionized water.Table (4).

Table (4): Duncan[a] Multiple Analysis Range test for tested groups after pH cycle.

Duncan[a] Groups	N	Subset for alpha = 0.01 1	2
C	10	190.1150	
VL1	10		255.8940
L1V	10		260.3090
Sig.		1.000	.178

***highly significant difference at p≤ 0.01**

Table (5) showed that the percentage of surface microhardness loss (SML%) for all groups was calculated according to equation: SML%=SMH2 –SMH1 / SMH1 × 100 (SML%: the percentage of microhardness loss, SMH1: surface microhardness at baseline, SMH2 : surface microhardness after PH cycle.)

Table (5): The percentage of surface microhardness loss of all tested groups.

Groups	**SML%**
De-ionized water	**38.75 %**
Varnish +Er,Cr:YSGG Laser	**16.55%**
Er,Cr:YSGGLaser + Varnish	**15.12%**

IV. DISCUSSION

Dental caries is a chronic infectious and communicable disease caused by specific bacteria, mainly mutans Streptococci which metabolize acid-related sugars that, over time, weaken the dental structure through demineralization[19].

The efficacy of topical fluorides in general and fluoride varnishesin particular regarding the reduction of dental caries has been reportedwidely [20].

The caries prevention effect of fluoride is owing to the development of acid-resistant fluorapatite crystals, which inhibit demineralization and enhance tooth structure remineralization. Various approaches incorporating the topical use of calcium and phosphate in order to increase effectiveness, including varnish-containing functionalized tri-calcium phosphate (fTCP) [21].

Laser induced enamel resistance is a novel technique for caries prevention [6].Er,Cr:YSGG (wavelength: 2.78 µm) has a large hydroxyapatite absorption [6].Controversial topics are present considering the optimum energy range for caries prevention application of erbium lasers. Laser photothermic effect will melt and fuse crystals from hydroxyapatite so that more acid resistant enamel will be generated [22]. Studies claimed that caries prevention should include the sub-ablative energy density [23].

In the current study, Er,Cr:YSGG laser beams were applied at energy density in experimental groups of 2.8 J/cm^2 and power of 2.5 watt [7]. Also, we used 600 µm diameter laser tips to achieve greater energy density with low laser power [24]. The quantity of pulses should be adequate, but the total energy transferred must be held to a minimum to prevent pulpal damage

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[7].While the frequency value for erbium lasers is not agreed, 20 Hz in this study was used as in the previous studies[12]. Excess water in the region through laser therapy has been shown to increase the potential for ablation and indeed induce the porosity of the tooth surface and hence the diffusion of acids into deep layers[25].Therefore, we chose laser irradiation without cooling of water in our research.

However, it is necessary to investigate the clinical efficacy of professional functionalized tri-calcium phosphate containing varnish combined with Er,Cr:YSGG laser in preventing of dental caries. In addition, a comparisonbetween the preventive effects of these techniques has not been clearly identified before on the teeth of people of Iraq. Therefore, evaluating the effectiveness of combination ofEr,Cr:YSGG laser and functionalized tri-calcium phosphate containing varnish (fTCP) as a pretreatment and post treatment strategy in increasing the caries resistance to demineralization is the aim of our study.The effect of that combination on human permanent enamel teeth was studied in vitro using Vickers microhardness test.

As enamel softening is a clinical characteristic of caries, laser induced caries prevention can also be assessed by means of a microhardness measurement. Therefore, microhardness test has been used in this analysis because it is known as a straightforward, more precise and less exhausting tool [8]. Demineralization and remineralization studies believe that SMH indentations can be regarded as non-destructive and reasonably fast process. A strong association between enamel microhardness and the loss of minerals was estimated in carious lesions [26].

According to the current study, the descriptive statistics and ANOVA Test demonstrated the difference among the tested groups at baseline and after pH cycle and noticed that there was no significant difference before pH cycle, while after the addition of the test materials and introduction in to the pH cycle, there was a high significant difference among tested groups as further noticed at Duncan[a], a Multiple Analysis Range Test for test groups after pH cycle, which illustrated that the groups of L1V and VL1 had the least reduction in the surface microhardness mean value without any significant difference between these groups, indicating that these groups will effectively protect the enamel of permanent dentition.

A few studies have tested the effects of combination of fluoride application and Er,Cr:YSGG laser irradiation on demineralization prevention of permanent teeth enamel [13,24]. In addition, the use of fluoride therapy and various types of lasers together could lead, according to previously mentioned studies, to a higher resistance of enamel compared with the use of fluoride or laser alone. In the literature, some inconsistencies regarding the order of fluoride application and laser irradiation can be found.Tagomori and Morioka (1989) found that laser irradiation with a subsequent application of fluoride can lead to greater intake of fluoride in enamel than when reversing the order [27]. Alternatively, laser irradiation of the fluoride covered enamel could causethe enamel's outer layer to melt and fluoride ions to bind to the hydroxyapatite crystals with the formation of fluorapatite crystals that are more resistant to acid than hydroxyapatite[14].Jenget al., (2013) also examined the effectiveness of this combination by applying fluoride compounds prior to irradiation. He found that the mechanical properties of the calcium fluoride-like deposits have improved [28]. Vieira et al., (2015) have the same findings and found that it was possible for hydroxyapatite crystals to convert directly into fluorapatite crystals using laser irradiation in the presence of fluoride [29]. In contrast, in the present study there was no significant difference in the microhardness according to the order of treatment when Er,Cr:YSGG0.25watt (2.8 J/cm^2) laser irradiation with functionalized tri-calcium phosphate varnish was used. Statistically, Er,Cr:YSGG (0.25watt) combined with functionalized tri-calcium phosphate varnish either before or after treatment provide thesame benefit to the enamelresistance against demineralization. Similar findings were obtained by Razeghiet al., (2018) who showed no significant difference in decreasing groups microhardness according to the order of treatment [12].

Overall, the diversity of results from different studies is likely due to variations in irradiation parameters, laser settings, demineralization solutions, fluoride agent type and demineralization measurement methods used in various studies. The form of laser and laser parameters like power and fluency appeared to be more significant than the order of the application of fluoride and laser irradiation[30].

Additional studies are needed to completely characterize the surface and chemical changes in enamel following combination of laser and Clinpro White varnish treatment.

V. CONCLUSION

The impacts on enamel resistance to demineralization and reducing its solubility seem to indicate that there is no difference in using Er,Cr:YSGG laser at 0.25watt as a pretreatment and post treatment for Clinpro White varnish.

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