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The Impact of Application of Clinpro White Varnish and Er,Cr:YSGG Laser Irradiation on Resistance of Enamel to Caries: An In Vitro Study

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ABSTRACT
Objectives: This study aims to investigate the efficacy of Clinpro White varnish and Er,Cr:YSGG laser in preventing of enamel caries.
Materials and Methods: Freshly extracted 30 sound upper first premolars teeth were divided into three groups, each group of ten samples. Group (C): only washed with deionized water. Group (V): Clinpro White Varnish. Group (L2): Er,Cr:YSGG laser (0.75 watt, 8.5 J/cm^2, 20 Hz, 11% air, 0% water) was irradiated. 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 a decreasing in surface microhardness in all groups due to the demineralization, but the least reduction in surface microhardness belonged to the group of Clinpro White varnish and group of Er,Cr:YSGG laser with no significant difference between them.
Conclusion:Er:Cr:YSGG Laser at laser power of 0.75 wattis indicated on decreasing the loss of enamel hardness with comparable effects to Clinpro White Varnish application.
Keywords: Er,Cr:YSGG Laser, Enamel Demineralization, Clinpro White Varnish, Microhardness.

I. INTRODUCTION

Dental caries is an infectious illness; carbohydrates fermentation by bacteria in the mouth is the basic procedure for caries development, which results in the production of organic acid. The mineral content of the tooth, calcium and phosphate in particular, can be dissolved by the action of these hydrogen ions that was produced by bacteria. This process known as demineralization. Preventing the progression of demineralization is possible, but when sufficient treatment is not provided, progressive destruction of the teeth will present [1].

Various preventive approaches are needed for the prevention of dental caries. Fluoride varnishes have showed significant clinical success in dental caries reduction in addition to its easy application and high patient acceptability [2]. On the other hand, to avoid demineralization, fluoride alone is not adequate. To maintain the structural integrity of hydroxyapatite crystals, the existence of bioavailable calcium and phosphate in the plaque fluid is also important. Therefore, alterations have been made to include calcium and phosphate ions within varnish structure in atrial to further enhance its effectiveness [3].Clinpro White Varnish with functionalized tri-calcium phosphate, or fTCP, is a “smart” calcium phosphate device that permits calcium and phosphate ions reserve to the teeth and acts synergistically with the addition of fluoride to improve efficacy [4].

Situations such as patients with compromised motility, withpoor dietary and oral hygiene, or those that use orthodontic devices still need strategies that offer a long-term preventive effect [5]. One of these approaches is to improve the resistance of enamel throughout the application of laser. The method of laser application to boost enamel resistance is a controversial protective technique which lasts for over 50 years with numerous in vitro studies on high density lasers [6]**.** Erbium lasers (Er:YAG and Er, Cr:YSGG), which are hard tissue lasers, occurred more recently than other kinds of laser. Since enamel contains high amount of water and inorganic content (86% inorganic content, 12% water and 2% lipid and protein) [1], water and hydroxyapatite have the ability to absorb high quantities of erbium lasers (Er:YAG and Er,Cr:YSGG) which results in thermal changes in the enamel. Thermal changes can cause morphological and/or chemical alterations in the structure of enamel [7].

The aim of the present in vitro study was to appraise the impact of the Er,Cr:YSGG laser andClinpro WhiteVarnish on enamel resistance to demineralization using artificial demineralization. The effect was studied in vitro using Vickers microhardness test.

II. MATERIALS AND MEHODS

Ethics statement:

This study protocol was performed in vitro, approved 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. Thirty sound upper first premolars with an intact buccal enamel surface were collected from patients aged (16-25) years old who got orthodontic treatment in private clinics in Basra. The teeth were visually examined, and only sound teeth (those with no visible signs of caries) were included in the study. The teeth with caries, restorations, discoloration, enamel developmental abnormalities, white spot lesions, fluorosis were excluded from the study.The extracted teeth were washed, and all deposits were removed, then rinsed with deionized water and stored in a 0.1 % thymol solution to prevent dehydration and the development of bacteria until they were used in fewer than 7 days [8]. After smoothing the teeth with non-fluoridated pumice, the remaining roots were cut 2mm below the cemento-enamel junctions with a straight diamond bur using copious irrigation to avoid harming the enamel, and the crown pulp was removed with an excavator. All specimens were inspected for cracks or enamel defects using a Zumax dental microscope and then embedded in chemically cured resin in plastic rings [8]. Each ring had a single tooth that was fixed in the center of the ring's upper surface, exposing the tooth's buccal surface. The middle third of the crown's buccal surface was marked using a caliper by measuring the distance between the cemento-enamel junction and the tip of the cusp, 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 except for a (4×2mm^2) window of uncovered enamel [1]**.** After drying the samples, the tapes were removed, exposing the enamel surface.

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 (0.75 W, 8.5 J/cm^2, 20 Hz, 11% air, 0% water) [9] .

The MZ6 tip was chosen for the handpiece's focal area with a beam diameter of 600 µm in order to avoid overheating of the tooth pulp [1]. To ensure a constant spot size throughout the irradiation procedure, an endodontic file fitted with a rubber stop was attached to the fixed handpiece at 1 mm from the enamel surface [10]. Furthermore, according to Almaz et al.,(2021) study, water cooling is not always needed [1]. The repetition rate was set to 20 Hz in accordance with the study by Ulusoyet al.,(2020) [8]. The irradiation parameters for the laser category were clearly illustrated in table (1). The samples were irradiated once for ten seconds by gently rotating the specimens horizontally by hand while holding the handpiece in its handle to guarantee adequate irradiation and coverage the whole exposed sample surface [9].

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

Laser group	Power (W)	Energy density (J/cm2)	Repetition rate (Hz)	Irradiation time (s)	Water	Air
Er,Cr:YSGG (L2)	0.75	8.5	20	10	NO	11%

Experimental design of the study:

Each tooth was given a unique number between 1 and 30 for identification purposes, and these numbers were used to randomly assign samples to one of three study groups. 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 [11]. and then subjected to the caries prevention approach described below:

- **Group (C) (control group):**no agent was applied, only washed with deionized water.

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- **Group (V):**Clinpro White Varnish was applied on the window of specimens' surface for four 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[8].
- **Group (L2) (Er,Cr:YSGG laser with parameter of (0.75 W, 8.5 J/cm^2, 20 Hz, 11% air, 0% water):** was irradiated as explained previously on the window of specimens' surface in a scanning style.

Then, using the pH cycling model, we used a cycle of demineralization and remineralization to produce artificial caries in all treatment groups. Additionally, all treatments were done by a trained operator, and the evaluation was done blindly.

pH-Cycling:

By exposing the teeth samples to pH cycling, artificial caries was formed. The teeth samples were immersed in a Demineralizing solution containing ($CaCl_2$ 2.2mM, NaH_2PO_4 2.2mM, and acetic acid 0.05M, PH 4.5, adjusted with KOH 1M) for three hours [12], followed by twenty hours in a Remineralizing solution containing ($CaCl_2$ 1.5mM, $NaHPO_4$ 0.9mM, and KCl 0.15mM, PH 7.0). Teeth samples were briefly washed with deionized water in between solutions and immersed in artificial saliva composed of (NaCl 0.40, KCl 0.40, $CaCL_2$.2H2O 0.79, $NaH_2PO_4.2H_2O$ 0.78, NaS9.H_2O 0.005, $CO(NH_2)_2$ Urea 0.1, in 1000 ml distilled water, PH 7 (concentration G \L) [13] for 30 minutes at the end of the demineralization process and 30 minutes at the end of the remineralization process. Ten cycles were performed on the teeth, with each cycle was lasting one day (24 hours). The demineralizing and remineralizing solutions were replaced daily, and the artificial saliva was changed following each treatment [12]. Preparation of the chemical solutions was done by the researcher at the Marine Science Centre in University of Basra.

Microhardness assessment:

All specimens were evaluated for their microhardness on two stages: 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 calculating the microhardness. The load and time were persistentfor all samples. Three indentations were made on each sample's exposed labial enamel surface, and the average value was documented as the specimen's microhardness [14].This test was conducted at University of Basra, Collage of Engineering, Department of Mechanic.

VHN= (kg \mm2) = 1.854 × P \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, in addition to the numbers of the samples of tested groups at the baseline and after pH cycle. Based on the means values for tested groups after pH cycle, Clinpro White Varnish 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
	N	10	10
Clinpro White Varnish (V)	Mean	307.4290	251.0220
	Std. Deviation	7.75936	11.98158
	N	10	10

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Er,Cr:YSGGLaser (0.75 W) (L2)	**Mean**	**311.2350**	**245.1320**
	Std. Deviation	**8.19641**	**7.96299**
	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 the 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**	80.422	2	40.211	.453	.640
	Within Groups	2394.978	27	88.703		
	Total	2475.400	29			
AfterpH Cycle	**Between Groups**	22570.750	2	11285.375	126.352	.000
	Within Groups	2411.553	27	89.317		
	Total	24982.303	29			

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

Duncan[a] multiple analysis range test was done to further explain that there was a high 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 V group and L2 group had a highly significant resistance against microhardness loss and there is no significant difference between them while the least value of surface microhardness belonged to the control group where no preventive agent was applied as demonstrated in table (4).

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

Groups	N	Subset for alpha = 0.01	
		1	2
C	10	190.1150	
L2	10		245.1320
V	10		251.0220
Sig.		1.000	.175

***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 %
Clinpro White Varnish	18.34%
Er,Cr:YSGGLaser (0.75 W)	21.23%

IV. DISCUSSION

The prevalence of dental caries is ranging from 49 % to 83 % according to the latest Global Oral Health Data Bank report and dental cavities affect almost all age groups[15]. This performance and prevalence illustrate the dental caries assessments and stresses the importance of the interprofessional team in dental caries prevention [16]. Various methods incorporating the topical use of calcium and phosphate in order to boost effectiveness, including varnish-containing functionalized tri-calcium phosphate (fTCP) [17].

Laser stimulated enamel resistance is a novel technique for caries prevention[8]. laser photothermic effect will melt and fuse crystals from hydroxyapatite so that more acid resistant enamel will be generated [18].

The evaluation of the effectiveness of Er,Cr:YSGG laser with power of 0.75watt and Clinpro White Varnish in increasing the resistance of enamel to demineralization is the aim of our study.

Er,Cr:YSGG (wavelength: 2.78 µm) has a large hydroxyapatite absorption [8]. Controversial topics are present considering the optimum energy range for caries prevention application of erbium lasers. Some studies said that caries prevention should involve the sub-ablative energy density [9]. Whereas other researchers have suggested that greater caries resistance can be achieved with higher degree of laser power [19]. The value of ablation threshold depends on laser pulse length, tissue absorption depth through laser pulses and heat diffusion [1].

In the current study, Er,Cr:YSGG laser beams were applied at energy density of 8.5 J/cm^2 accordance to studies of Anaet al., (2012) [19] ; Razeghiet al., (2018) [9] andAlmazet al., (2021) [1]. Also, we used 600 µm diameter laser tips to achieve greater energy density with low laser power [20]. The laser frequency (Hz) should be established at a level that can be clinically effective and allow sufficient cooling between pulses at the same time. The quantity of pulses should be adequate, but the total energy transferred must be held to a minimum to prevent pulpal damage [1] .While the frequency value for erbium lasers is not agreed, 20 Hz in this study was used as in the previous studies[9].In some previous studies, the low energy intensity of laser as well as the use without water would avoid the ablation of the target tissue [9]. Therefore, we chose laser irradiation without cooling of water in our research. In this study we use the 10 second period in according toRazeghi et al., (2018) study [9].

The effect of Er,Cr:YSGG laser and Clinpro WhiteVarnish on human permanent teeth enamel's resistance to demineralization was studied in vitro using Vickers microhardness test.

Hardness is a mechanical property that allows the degree of mineralization of the tooth to be determined. It depends directly on the mineral content and the enamel prism crystalline. As enamel softening is a clinical characteristic of caries, laser induced caries prevention can also be evaluated by means of a microhardness measurement. Therefore, microhardness test has been used in this analysis because it is known as a simple and more accurate [21]. The overall protective effects of various preventive regimens can be assessed in the test of surface microhardness[22].

According to the current study, the descriptive statistics include means, standard deviations and the numbers of all study groups at baseline and after pH cycle. Based on the means values, the preventive effect of Er,Cr:YSGG laser irradiation andClinpro White Varnish was better than control group of deionized water.

ANOVA Test demonstrated the difference among the tested groups at baseline and after pH cycle and observed that there was no significant difference at baseline, 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 showed that Er,Cr:YSGG laser irradiation andClinpro White Varnish had a minimum reduction in the microhardness mean value with no significant differences between themand both of them were useful to protect the microhardness of enamel surface of permanent teeth in comparison with the control group of deionized water.

In Clinpro White Varnish Group, there was 18.34% loss in the percentage of microhardness as compared to the control group of deionized water which has 38.75 % loss in the percentage of microhardness after ph. cycle.Calcium fluoride (CaF_2) has been developed on enamel surfaces and fluoride was released to fluid levels after a professional application of fluoride with calcium and phosphate has been performed. This effect helps to reduce the demineralization of enamel.In addition, the dose-response effect between enamel mineralization and fluoride released through reserve concentrations of CaF_2, and subsequent inhibition of enamel demineralization was observed [7].The findings of present study have shown that topical application of varnish was effective in the prevention of demineralization process.The research of Alamoudiet al.,(2013) revealed that fTCP containing varnish had significantly higher SMH than unprotected teeth, and hence the protective effect was substantially higher [4].

Earlier studies found that the increase in laser power at sub-ablative threshold (8-13J/cm^2) would increase the superficial temperature and create more morphologically and crystallographic modified enamel including alpha-phase formation of tricalcium phosphate, beta- phase tricalcium phosphate and tetra calcium phosphate [23]. These changes may be the reason of enhanced overall demineralization resistance of irradiated enamel identified in previous study [24].In other words, Researchers have also attributed improvements in surface resistance to the photo-chemical effect of a laser by lowering the carbonate contents or partial decomposition of the organic matrix [19].These results of previous studies were similar to the present study , so, The microhardness of samples with laser power density of 8.5 J/cm^2 have increased compared with control samples and there was 21.23%loss in the percentage of microhardness in L2 group after ph.cycleas compared to control group of deionized water with 38.75%.Although, Apelet al.,(2004) indicated that 8 J/cm^2 energy density was not able to cause changes in surface hardness following an in-situ cariogenic challenge [24]. The difference may be associated to sample variations including total mass, hardness, thickness, hydration and other characteristics can lead to different results when the specimen interacts with lasers.

In the present study the benefitsaccomplished by laser radiation alone are not superior to those achieved by topical application of varnish, whereas laser at power density of 8.5 J/cm^2 had statistically similar effects to fTCP varnish.

The percentage of surface microhardness loss of fTCP varnish group was 18.34% in compared with L2 group which had 21.23% of surface microhardness loss. This difference could be due to the laser radiation effect formed in the production of fine irregularities and cracks in enamel surfaces that favored enamel brittleness. These cracks serve as the base for acid attacks and cause enamel to be fragile, resulting in a reduction of SMH. These findings were in agreement with Ana et al., (2012) results which showed that the benefits achieved with laser radiation alone are not higher than those with topically treated fluoride treatment but the laser that applied at 8.5 J/cm^2 had similar effects to acidulated fluoride [19].

Additional research is necessary to completely characterize the surface and chemical changes in enamel following laser and/or clinpro whitevarnish treatment. The experimental findings of this study showed promising opportunities with several clinical limitations.

V. CONCLUSION

The Er:Cr,YSGG Laser at energy density of 8.5 J/cm^{2}and power of 0.75 watt is indicated on decreasing the loss of enamel hardness with similar effects to clinpro white varnish application.

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