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Influence of Er,Cr:YSGG and Diode Lasers on The Microhardness of Enamel at Fissure area: (An in vitro Study)

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Abstract

Aims: To assess and report the microhardness incidence in fissure system of the human dental enamel next Er,Cr:YSGG laser and Diode laser applications in order to examine anti-cariogenic impact and to compare the obtained results between the two laser types. **Materials and Methods**: Forty extracted impacted lower third molars teeth used in the experiment that should have an obvious occlusal fissure. Intact lower third molar were collected, which were free from caries. Teeth roots separated from crowns ,then the crowns were separated bucco-lingually into two equal parts which were applied in 4 test groups included control group without any treatment, acid group only immersed in demineralizing solution, Diode laser group in which samples subjected to Diode laser (at wavelength 980nm, power 5 watt, mode P3, the time was 30 second) then immersed in demineralizing solution PH 4.4 for 96 hr., and Er,Cr:YSGG laser group in which samples subjected to Er,Cr:YSGG laser (wave length 2780nm, pulse energy 20 Hz with pulse mode (H-mode), time 10 second) then immersed in demineralizing solution pH 4.4 for 96 hr. **Results**: The mean microhardness was significantly different from each other at $p \leq 0.01$. And Er,Cr:YSGG laser had mean surface microhardness significantly higher than other groups then Diode laser group. **Conclusions**: Both types of lasers increased microhardness of enamel at fissure area, and Er,Cr:YSGG had better effect than Diode laser.

الخلاصة

الأهداف: تهدف الدراسة الى تقييم وتعيين صلادة السطح التي تحدث بطبقة المينا للشقوق الموجودة على سطح الاطباق بعد استخدام ليزر الدايود و ليزر الإربيوم لفحص تأثيرهم لمقاومة التسوس ومقارنة النتائج بين الدايود ليزر والاربيوم ليزر.**المواد وطرائق العمل**: تم استخدام اربعين ضرس عقل سفلي واضحة الشقوق على سطح الاطباق. ضروس العقل التي جمعت كانت سليمة وخالية من التسوس. تم فصل الجذور عن التيجان وقُطعت التيجان الى نصفين من السطح الخدّي الى اللساني بالتساوي ثم وُزّعت عشوائيا بين اربع مجاميع، مجموعة السيطرة خالية من العلاج، مجموعة الحامض غُمرت بالحامض فقط (درجة حامضيته 4.4 لمدة 96 ساعة)، مجموعة ليزر الدايود التي عُرّضت فيها العينات للدايود ليزر (عند طول موجي 980 نانومتر وطاقة 5 واط ومدة التعرض 30 ثانية) وبعدها غُمرت في الحامض، ومجموعة الاربيوم ليزر عُرّضت فيها العينات للإربيوم ليزر (عند طول موجي 2780 نانومتر ونبض 20 هرتز لمدة 10 ثواني) ثم غُمرت في الحامض. **النتائج**: اختلف معدل الصلادة بدلالة احصائية عالية بين المجاميع الاربع وحصل الإربيوم ليزر على المعدل الاعلى للصلادة. **الاستنتاجات**: كلا الدايود ليزر والاربيوم ليزر زادا من صلادة المينا بالشقوق بسطح الاطباق ولكن الاربيوم افضل من الدايود.

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INTRODUCTION

It is well known that dental caries is the most prevalent oral disease and considered as a public health problem, Occlusal pits and fissures service the stagnation of food debris and bacteria and assist the initiation of tooth decay.[(1)]

The anatomy of pits and fissures, which supply shelter for microorganisms, prevents adequate plaque control, and cause insufficient hygiene on the occlusal surfaces. These make the posterior teeth exposed to carious lesions.[(2)] And research was stated that about 90% of dental carious produce from fissures and pits in the occlusal surfaces of the posterior teeth.[(3)] Recently , more interest has been gotten to preventive dentistry, the purpose of which is to prohibit dental caries and decrease the possibility of caries. Several procedures are obtainable for the avoidance and decrease of dental caries, including nutritional alteration, systemic and topical fluorides, pit-and-fissure sealants, sugar substitutes, caries vaccines, etc. Pit-and fissure-sealing is one of the most highly suggested and generally known preventive procedures, particularly in newly-erupted permanent molars for preventing caries.[(4)]

Marginal loss and wear are the most noticeable problems of the conventional sealing materials, leading to rapid exposition of the formerly sealed areas. Therefore, an acceptable bond not achieve for fissure sealants as outcome of the lack of tag creation next short etching of the prismless structure lines the fissure system.[(5)]

As related to these drawbacks of fissure and pit sealants, investigation was organized to laser and its favorable result on the dental enamel surface of teeth. Laser use in dentistry has been a study attention for the past 30 years and has lately increased in acceptance. The dental hard tissue irradiated with laser are the less vulnerable to acid attack and caries because of decreases the carbonate: phosphate ratio, changes the calcium: phosphorus ratio and leads to the development of more stable and less acid-soluble compounds.[(6)]

The laser is capable of changing the crystalline structure, the permeability, and progressing a raise in resistance to demineralization by the dental enamel. The effect is related to the chemical-physical changes made on enamel by laser.[(5)]

This study was conducted to assess the microhardness incidence in fissure system of the dental enamel in human following Er,Cr:YSGG laser and Diode laser applications in order to examine anti-cariogenic impact and to compare the obtained results between the two laser types.

MATERIALS AND METHODS

Teeth Sample Collection and Criteria: Forty extracted impacted lower third molars teeth from individuals at age range between 20-25 years used in the experiment that should have an obvious occlusal fissure. Intact lower third molar were collected, which were free from caries, no enamel hypoplasia or developmental anomalies, no cracks, wears or fractures.

The Experimental Design of the Study: The total number of teeth sample in the study was (80) samples. The samples were randomly distributed into 4 groups exposed to various type of treatment. Group 1: the included samples were not received any type of treatment, Group 2: the included samples were submerged in a demineralization solution, Group 3: the included samples were received Diode laser prior to its immersion in the demineralization solution, finally Group 4: the samples were received Er,Cr:YSGG laser irradiation before to its immersion in the demineralization solution.

Sample preparation: All soft tissues were removed by scaling and teeth were polished with super-fine fluoride free pumice using rubber cup. Teeth were stored in thymol solution (0.1%, pH7.0) to avoid bacterial growth and were kept in a refrigerator at 4 °C till use.[7] Utilizing diamond low speed disc teeth roots cut from crowns and crowns were divided bucco-lingually to two equal parts which were applied in 4 test groups.

Diode Laser Application: Teeth samples in group (3) were exposed to diode laser at wavelength 980nm and the power was 5 watts,[8] mode P3, the time was 30 second and the tips used were e4 (Epic x 4mm length, 0.4mm diameter).

Er,Cr:YSGG Laser Application:
Er,Cr:YSGG Laser Application: Laser parameter applied in the application wave length 2780 nm, pulse energy 20 Hz with pulse mode (H-mode), time 10 seconds. The power Er, Cr: YSGG laser applied in the current study was (0.75 W). This power was established on prior studies which likened other Er,Cr:YSGG power values or with other types of lasers. Results discovered the best effects concerning acid resistance enhancement in 0.75 W power was gave.[11,12] Water and air flow were set to (40%,60%) respectively.[11] MZ6 tips with (6mm) length and (0.6mm) diameter were used for samples irradiation and Irradiation distance 1mm from enamel surface.

Sample Mounting in Acrylic Ring: Plastic ring were cut and prepared so that, the upper and lower sides were flat and parallel to each other (12mm diameter×14mm depth). Embedding the tooth in epoxy resin with the cut side exposed then polished one by one with a fine grit silicon carbide papers (600-,800-, and 2400 grit). Lastly, all samples were washed with deionized water and kept till the immersion in demineralized solution.

Immersion in Demineralized Solution: All samples except control group were immersed in demineralizing solution for 96 hr. (4 days) at 37 °C which consist of (2.2 mM) KH_2PO_4, (2.2 mM) $CaCl_2$, and acetic acid (0.05 M), with pH (4.4) modify by (1 M) KOH.[12] Then all samples were picked up from the solution after 96hr. and washed with deionized water.

Microhardness test: The mechanical properties of the teeth samples were measured using a Vickers microhardness machine. Measures were achieved proximally at the lateral sides of the fissure depth and at the depth of the fissure. The long axis of the knoop diamond perpendicular on the depth of the fissures and at the inner enamel surface laterally in that manner indentations were completed.(5) The microhardness numbers were calculated from the length of the indentation on the enamel, indentation length was determined microscopically with 600X magnification. Measuring of surface hardness accomplished at the Technical institute/ University of Mosul. The enamel surface of samples was subjected to 1 kg load for 15 seconds, the load and time were constant for all samples. The size of the indent was resolute optically by measuring the two diagonals of square indent The Vickers number (HV) calculated using following formula:(13)

$HV=1.8544L/d^2$

L is calculated in Kg f and d (mm) is equivalent to the distance of the diagonal measured from angle to angle on remaining impression in the enamel sample surface.

Statistical Analysis: Data were analyzed utilizing SPSS program which involved descriptive statistics which included mean and standard deviation for each group, Independent t-test Sample: This test was utilized to evaluate the surface microhardness between specimen groups ofEr,Cr:YSGG laser group and diode laser group, ANOVA test (One Way Analyses of Variance) and Duncan's Multiple Range Test were employed to observe the significant variances between the tested samples. The statistical results were regard as significant at $p \leq 0.01$.

RESULTS

The descriptive statistics that contains the mean, number, standard deviation and range for all groups are listed in table (1). The mean values of enamel surface microhardness in fissures are compared by one-way analysis of variance (ANOVA) test, and the results revealed that there were a highly statistically significant differences within and in between groups at $p \leq 0.01$ as shown in table (2).

Duncan’s Multiple Range test display that the mean microhardness for control group, acid group, diode laser group and Er,Cr:YSGG laser group are significantly different from each other. Er,Cr:YSGG laser had mean surface microhardness significantly higher (401.6550) than other groups, Diode laser had mean surface microhardness (378.5150), Control group had mean surface microhardness (374.2050) while acid groups had lesser mean on surface microhardness (315.2700) as shown in figure (1). The analysis of variance of t-test used for Er,Cr:YSGG group and Diode laser group. Er.Cr:YSGG group show more enamel surface microhardness in fissures than diode laser group at ($p \leq 0.01$) as illustrated in table (3).

Table (1): Descriptive statistics of surface microhardness measurements among tested groups.

Groups	Surface Micro hardness Mean	N	Std. Deviation	Range
Control	374.205	20	10.12041	35.4
Acid	315.27	20	8.73837	29.8
Diode	378.515	20	10.84519	38.2
Er,Cr:YSGG	401.655	20	9.25178	28.6
Total	367.4112	80	33.46522	115.3

Table (2): ANOVA test of surface microhardness between and within tested groups at $p \leq 0.01$.

Microhardness	ANOVA Test Sum of Squares	df	Mean Square	F	Sig.
Between Groups	81215.85	3	27071.95	283.479	0.000
Within Groups	7257.912	76	95.499		
Total	88473.77	79			

P≤ 0.000 highly statistically significant

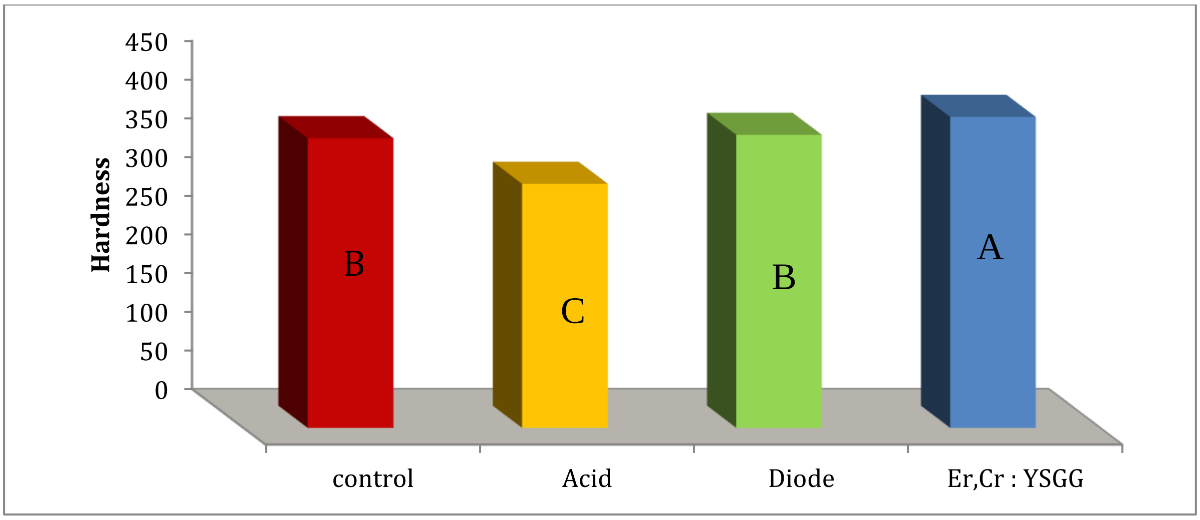

Figure (1): Duncan's Multiple range test of surface microhardness measurements for Control group, Acid group, Diode laser group and Er,cr:YSGG laser group.

Table (3): The t-test analysis to determine significant difference between laser groups (Diode and Er,Cr:YSGG) in surface microhardness.

Microhardness	N	Mean	t-value	sig	Std. Deviation	Std. Error Mean
			t-test			
Diode	20	378.51	-7.259-	0	10.84519	2.42506
Er,Cr:YSGG	20	401.66		0	9.25178	2.06876

** highly statistically significant at $p \leq 0.01$.

DISCUSSION

The in vitro studies presented the involvement of two types of lasers (Er,Cr:YSGG lasers and diodes) that were capable of strengthening the dental enamel against demineralization by acid (analogous to those of caries or acids similar). Actually, the enamel irradiated with laser light, after being exposed to acid solutions for demineralization, presented a lesser loss of surface hardness (microhardness tests), a lesser minerals loss (spectroscopic analysis), and a lesser average depth of cavity lesions (light polarized microscope evaluations and scanning electron microscopy) compared with untreated enamel.[9,14]

Er,Cr:YSGG laser with(2780 nm) wavelength shows highly absorption in hydroxyapatite and water, the highest ingredients of the dental enamel. The enamel absorption coefficient about to 50 mm−1. As result of that the penetration of the laser beam in enamel about 21 μm from the surface and as a consequence, the absorption of Er,Cr:YSGG laser irradiation mainly in the superficial layers of enamel, that consider advantageous for the dental pulp safety . At this treatment it is essential for the laser beam not to ablate the treated enamel surface, but only to alter it chemically or/and morphologically so as to stimulate more surface resistance to erosive activity.[15]

The calculation of surface’s microhardness is an appropriate method which was carried out using Vickers Hardness Measuring method. The benefit of this method is high accuracy and quantitative measurement capability.[16] Enamel surface microhardness is indicative of enamel mineral content and microhardness measurement has acceptable sensitivity to measure enamel resistance to demineralization. Furthermore, a significant connection was stated between mineral loss in carious lesions on teeth and the surface microhardness of enamel.[17] The results of the current study were displayed a significant raise in enamel microhardness after exposure to Er,Cr:YSGG laser then to acid challenge which mean value approximately (401.6) kg/mm^2 more than enamel exposed only to acid without laser (315.2) kg/mm^2, or enamel not subjected to laser and acid challenge (374.2) kg/mm^2, this agree with Kumar *et al*., (2016)[18] who study concluded that Er,Cr:YSGG laser application on enamel surface raises the surface microhardness thereby increasing the enamel resistance to caries. And agree with Pavithra *et al*., (2016)[19] who reported that microhardness value obtained after erbium laser irradiation was higher than the microhardness of unlased pits and fissures. The use of diode laser for a hard tissue procedure can be justified from the information given by Romanos and Nentwig (1999)[20] whose observed that the penetration depth of a diode laser at 980 nm wavelength was lesser compared to erbium and Nd: YAG laser. The lesser penetration depth resulted in amplified energy deposition at the surface, melting, and recrystallization of the enamel structure, improved microhardness and recrystallization of enamel structure.

The diode laser was more effective against enamel acid dissolution than no treatment[21,22]. Saafan *et al.*, (2012)[23] reported that diode laser, a traditional soft tissue laser, induced enamel fusion in the pits and fissure with no harmful effects on the dentin and pulp. Its low absorption coefficient in enamel causes fast increase in surface temperature resulting in melting of enamel crystals and recrystallization.

The results of the current study were presented a significant raise in enamel microhardness after irradiation by Diode laser about (378.5) kg/mm^2 as compared to control group about (374.2) kg/mm^2 and acid group about (315.2) kg/mm^{2}which subjected to acid without laser radiation and this concurs well with previous study by Pavithra *et al.*, (2016)[19] who reported that microhardness values obtained after diode laser irradiation was also higher than the unlased pits and fissures.

CONCLUSION

Based on results of the existing study, it can be concluded that microhardness of a lased enamel surface is higher following Er,Cr;YSGG laser treatment when compared to and Diode laser treatment. However, though Diode laser is traditionally viewed as a soft tissue, it has produced a significant raise in the enamel microhardness compared to the control in the study. Hence, it has dual advantage of lesser cost and being safer to the dentin-pulp complex. However, further research is essential towards this end.

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