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EVALUATION OF ER,CR: YSGG LASER IN FLAPLESS FUNCTIONAL CROWN LENGTHENING (A Randomized Controlled Clinical Trial with Microbial Analysis)

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ABSTRACT

Background: Several studies have described the benefits of LASER when used for crown lengthening (CL). However, there has been a lack of clinical trials comparing surgical functional crown lengthening (FCL) and LASER flapless CL. The aim of this study was to compare both methods together in terms of soft-tissue wound healing, gingival marginal stability and sub-gingival bacterial load reduction.

Subjects and methods: Twenty patients with eligible teeth for FCL were recruited and randomly allocated to either; **Group I:** 10 posterior teeth indicated for FCL received closed flap laser assisted CL utilizing (Er, Cr:YSGG) laser or **Group II:** 10 posterior teeth indicated for FCL received open flap conventional surgical technique. Soft tissue healing was evaluated on the 7th day,45th day and after 3 months. Gingival marginal stability was evaluated immediately after surgery and after 3 months. Sub-gingival bacterial load was assessed at base-line, on the 7th day and after 45 days.

Results: Laser induced faster and significantly better soft tissue healing at 7 days but insignificantly less frequent stable gingival margin after 3 months when compared to conventional surgery. Laser induced significantly higher percentage reduction of bacterial load in the gingival sulcus than conventional surgery after 7 and 45 days.

Conclusion: Closed flap laser-assisted CL using Er, Cr: YSGG laser can be used as an alternative procedure for conventional surgical FCL with better and faster healing and bacterial disinfection.

KEYWORD: Badly-broken teeth, Biologic width violation, Crown lengthening, Erbium Laser, Flapless Surgery

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INTRODUCTION

Dentists face the problem of teeth with subgingival caries or fractures or both on daily base causing the dilemma of whether to extract those teeth and replace them with implants later on or to restore them because a sound tooth structure should be exposed for the restorative therapy [1].

The biologic width was first described by Gargiulo as the dento-gingival junction of the tooth and was found to be of average 2.04mm in length. This complex consists of connective tissue attachment of mean value 1.07mm and epithelial attachment of mean value 0.97mm[2]. Later Vacek studied human cadavers to investigate the dimensions of this dento-gingival complex and reported mean values of 0.77mm for the connective tissue and 1.14 mm for the epithelial attachment [3].

Violation of biologic width while attempting to restore badly broken-down teeth is well known to cause chronic progressive inflammatory process at the restoration margin, bleeding on probing (BOP), clinical attachment loss (CAL), alveolar bone loss (ABL) with pocket formation or gingival recession. This inflammatory process is formed because the body is trying to create a space for reformation of the attachments after they have been encroached [4].

Gunay demonstrated that placement of the restoration margin within the biologic width is harmful for the periodontal tissues. A 3mm distance from the alveolar bone crest (ABC) to the margin of the restoration is sufficient to decrease the possibility of periodontal destruction [5]. One of the procedures readily used to expose sound tooth structure without biologic width violation is the functional crown lengthening procedure. Functional crown lengthening is defined as a surgical procedure designed to increase the extent of the supragingival tooth structure for restorative or esthetic purposes by apically positioning the gingival margin, removing supporting bone or both [1,6].

The conventional FCL is performed by surgical excision of small part of the gingiva with the lancet, or apically positioning of the gingival margin, full thickness flap elevation with recontouring of the alveolar bone by a surgical bur, hand chisels or piezoelectric cutting device should be performed to create a biological width space [7].

On the other hand, although it is a simple treatment, it is still a surgery that involves a flap elevation with suturing, thus the patients always complain from post-operative pain and discomfort. Also, it requires a healing period before the final restoration could be placed. Final healing of the tissues to reach maximum vertical height may take up to 3 months after surgery. Others suggested that periodontal tissues need 6 months as a healing period to be stable [8,9].

Er, Cr: YSGG laser is 2790-nm laser with a high absorption rate into water molecules thus remove mucosal and bone tissues by a traumatic cool thermo-mechanical ablation mechanism [10].

Erbium laser can remove both soft and hard tissues. The use of Erbium laser in periodontal surgeries such as gingivectomy and implant exposure offered many advantages as simplicity, less bleeding, less time needed, no sutures and less post-operative discomfort, as well as shortening the healing time period to about 2 weeks and providing more stable results [11]. Moreover, erbium lasers have anti-bacterial effects and can reduce the quantity and modify the species of the periodontal pathogens. However, the blind flapless laser assisted technique may result in bone irregularities and injury to adjacent tooth surfaces as described in a single animal study [11,12].

The ability of lasers to perform soft and hard tissue crown lengthening has been described in several published case reports and case series. In all these case reports and case series, the laser offers many advantages like producing less pain, reasonable bleeding, less post-operative complications, sterile wound cut and accelerated healing time compared to conventional method [13-17].

Based on previous review of literature we hypothesized that erbium laser assisted flapless functional crown lengthening may offer better alternative to conventional surgical crown lengthening procedure regarding patient comfort, soft tissue healing and bacterial load reduction but to the extent of our knowledge, there is only two published researches that compared both methods (one was performed on animals and the other on humas) [11,18]. So, we designed our study as a randomized controlled clinical trial to fill this gap of knowledge.

The aim of the present study was to evaluate the use of Er, Cr: YSGG laser with closed flap approach versus open flap conventional surgical approach in performing FCL procedure in terms of soft tissue wound healing, gingival marginal stability and subgingival microbial changes.

SUBJECTS AND METHODS

Study design:

A randomized controlled clinical trial in which twenty patients indicated for FCL were recruited from the outpatient clinic of the department of oral medicine, periodontology and oral diagnosis, Faculty of Dentistry, Ain shams University. The protocol of this study was reviewed and approved by the ethical committee of the Faculty of Dentistry, Ain Shams University (FDASU-Rec091903). This study was conducted in accordance with Helsinki Declaration of 1975, as revised in 2013. This study was conducted after receiving an ethical clearance from the Research Ethics Committee of Faculty of Dentistry, Ain Shams University, that the study follows the guideline of research ethical committee. The study protocol was explained in details to all patients along with complications expected after surgery such as infection, inflammation, swelling and even tooth extraction. The patients understood the purpose of this study then signed an informed consent.

Power analysis was performed to have adequate power to apply statistical test of the research hypothesis. Assuming an alpha (α) level of 0.05 (5%), a beta (β) level of 0.05 (10%) i.e power= 95%, and an effect size (d) of (1.54) according to the results of the reference paper used for analysis (18). The predicted sample size (n) was a total of twenty samples: Ten for each group. Sample size calculation was performed using G*power (version 3.1.9.22).

The participant was included or excluded according to the following criteria. Inclusion criteria: both genders within age range of 20-50 years. Single posterior (premolars or molars) badly broken down endodontically treated tooth with subgingival caries or subgingival margins located less than 4mm from crestal bone as evaluated by bone sounding and periapical radiograph (**7**). Sufficient amount of attached keratinized gingiva on the selected teeth and should maintain adequate amount ≥2 mm after surgery [19]. Good oral hygiene, patient compliance and good periodontal health of the involved teeth. Exclusion criteria: presence of any systemic disease or taking drugs that contraindicate oral surgery using predesigned questionnaire for medical history. Smokers, alcoholics or drug abusers. Pregnant or lactating females. Patients with previous surgery in the required area. Mobile teeth or teeth with compromised periodontium. Vulnerable groups (prisoners, mentally retarded, handicapped and orphans). Patients with previous anti-biotic therapy in the past 6 months.

Patients grouping:

Group I (Test group): included 10 patients indicated for FCL to receive closed flap laser assisted crown lengthening utilizing Er, Cr: YSGG laser **(Waterlase I plus, USA).**

Group II (Control group): included 10 patients indicated for FCL to receive open flap conventional surgical technique.

Treatment protocol:

Preoperative medications and instructions: All patients received professional scaling using ultrasonic scalers, manual scalers and hand curettes and were given oral hygiene instructions. Patients were checked for their oral hygiene measures after one week and those who did not follow the instructions were excluded from the study. Pre-operative radiograph and bone-sounding to evaluate amount of tooth destruction as shown in **Figure (1).**

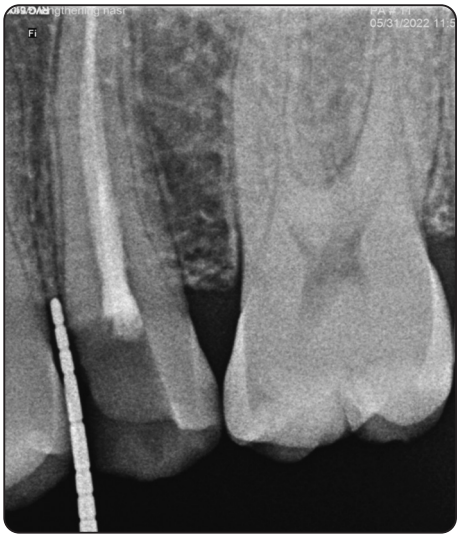

Fig. (1) A preoperative radiograph showing bone sounding of a badly destructed upper left second premolar

Treatment steps for Group I: Taking bacterial samples from the gingival sulcus by insertion of paper points (size 30) mesially, distally, buccally and palatally (lingually) until feeling a resistance for 30 seconds under cotton isolation**.** Then placement of these paper points in Eppendorf containing 0.5 ml phosphate buffered saline with PH 7.4. The samples are labelled and stored at -20 °C immediately until the analysis. Local anesthesia was administrated by infiltration technique both buccally and palatally or lingually. The level of the new gingival margin was determined using a Kirkland knife. Removal of the predetermined gingival tissue utilizing MGG6 laser tip as shown in **Figure (2)**.

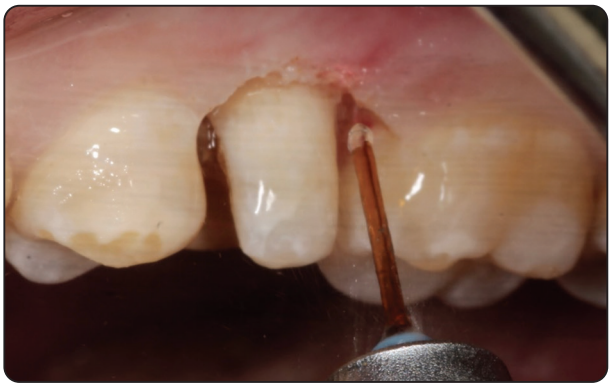

Fig. (2) A photograph showing the removal of the gingival tissue utilizing MGG6 laser tip.

The tip was moved from one side to another with 2-watt, 50-75 Hz, 40% water, 20% air and short pulse duration 100-150 msec. The gingival tissue is removed buccally, lingually or palatally and interdentally [20]. Post and core build-up under isolation utilizing rubber dam. Bone sounding to determine the position of the osseous crest relative to the restoration margin. Bone removal (Osseous ablation) utilizing MGG6 laser tip that is placed parallel to the long axis of the root with 4 watts, 20-30 Hz, 60% water, 20% air and short pulse duration 100-150 msec. The tip was held 1 mm from the osseous crest then advanced apically to remove bone. Bone recontouring with a sweeping motion and the tip moving from mesial to distal following CEJ through the sulcus until a 3mm was obtained from the osseous crest to the planned crown margin guided by a stopper. Each time after contouring the bone, the uniformity of bone on all the surfaces was evaluated using a periodontal probe. Bone was removed buccally, palatally or lingually and interdentally to preserve a positive architecture[20]. Radial firing periodontal tip was used to disinfect the sulcus in a crown-down motion with 2 watts, 24% air and 16% water for 20 seconds. No suturing was needed by the end of surgery, only applying pressure by a moist gauze for bleeding control and to adapt the tissues.

Treatment steps for Group II: Taking bacterial samples from the gingival sulcus by insertion of paper points (size 30) mesially, distally, buccally and palatally (lingually) until feeling a resistance for 30 seconds under cotton isolation**.** Then placement of these paper points in Eppendorf containing 0.5 ml phosphate buffered saline with PH 7.4. The samples are labelled and stored at -20 °C immediately until the analysis. Local anesthesia was administrated by infiltration technique both buccally and palatally or lingually**.** The level of the new gingival margin was determined using a Kirkland knife. Internal bevel incision was made 1-1.5mm from the gingival margin on both buccal and lingual or palatal surface including the papilla mesially and distally. Reflection of full thickness mucoperiosteal flap to expose the bone in all surfaces (buccally, lingually or palatally, mesially and distally). Sulcular incision was made both buccally and lingually or palatally to remove the gingival collar. Post and core build-up under isolation utilizing rubber dam. Bone was removed (ostectomy) with high-speed end cutting bur to create of space of 3mm of sound tooth structure between the alveolar bone crest (ABC) and the proposed crown margins in all surfaces for a healthy biologic width and for restoring the positive bone architecture. Smoothening of the interdental bone with bone file (Sugarman periodontal file). Suturing using modified vertical mattress suture with Vicryl 5-0 suture.

Temporary crowns were fabricated in treated teeth for both groups to prevent soft tissue creeping during healing. Impression was taken and sent immediately to the laboratory to fabricate a stent that was used as a guide for placement of the periodontal probe to monitor marginal tissue stability by measuring the space between the new gingival margin and a marking in the acrylic stent for reproducible measurement.

Post-operative instructions for both groups: Ibuprofen 400 mg. tablets were prescribed on need basis. Routine postoperative instructions will be given to the patients in a written form. Patients were given Wong-Baker faces pain rating scale template to record their post-operative pain. Patients were instructed to come to the clinic for follow-up and sutures removal (in group 2) after 7days. The patients were referred to the department of fixed prosthodontics to receive their final restoration after the required healing period.

Soft tissue healing: was assessed using healing Index of Landry, Turnbull and Howley (tissue color, bleeding on palpation, granulation margin, incision margin and suppuration) on the 7th day,45th day and 3 months (**figure 3**) after surgery [21].

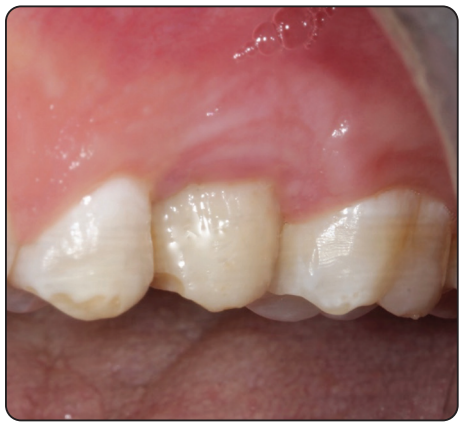

Fig. (3) A photograph showing follow-up of the healing after 3 months with the temporary crown.

Gingival margin stability: The position of gingival margin in relation to a reference point in the acrylic stent was evaluated immediately after surgery and after 3 months healing period [18].

Bacterial disinfection: Gingival crevicular fluid (GCF) samples were taken by paper points (size 30) to assess bacterial DNA copies (copies/ul) at base-line, on the 7th day and 45 days after surgery using real time PCR technique. GCF collected samples contaminated with saliva or blood were discarded[22].

Statistical analysis

Numerical data were explored for normality by checking the distribution of data and using tests of

normality (Kolmogorov-Smirnov and Shapiro-Wilk tests). Age, bacterial load and percentage reduction in bacterial load data showed normal (parametric) distribution while other numerical data showed non-normal (non-parametric) distribution. Data were presented as mean, standard deviation, median and range values. For parametric data, Student's t-test was used to compare between mean age in the two groups. Repeated measures ANOVA test was used to compare between bacterial load and percentage reduction in bacterial loads in the two groups as well as to study the changes by time within each group. Bonferroni's post-hoc test was used for pair-wise comparisons when ANOVA test is significant. For non-parametric data, Mann-Whitney U test was used to compare between the two groups. Friedman's test was used to study the changes by time within each group. Dunn's test was used for pair-wise comparisons when Friedman's test is significant. Qualitative data were presented as frequencies and percentages. Chi-square test or Fisher's Exact test was used for comparisons between the groups. The significance level was set at $P \leq 0.05$. Statistical analysis was performed with IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp.

RESULTS

I- Demographic data:

There were no complications and all the patients completed the study. There were 6 (60%) males and 4 (40%) females in group I. In group II, there were 4 (40 %) males and 6 (60%) females. The mean age was 35.3 in group I and 34.1 in group II. Regarding teeth types, there were 4 (40%) premolars and 6 (60%) molars in group I while there were 5 (50 %) premolars and 5 (50%) molars in group II. There was no significant difference between both groups regarding mean age, gender distribution and teeth types. ($P \leq 0.05$).

II- Clinical Outcomes:

Regarding the soft tissue wound healing:

After seven days, there was significant difference between soft tissue healing in the two groups. Group I showed better healing compared to group II. (p=0.048) There was no significant difference between soft tissue healing findings in the two groups after 45 days (p=0.087) as well as after three months (p=0.350) as shown in **Table (1)**.

TABLE (1) Frequencies (n), percentages (%) and results of Fisher's Exact test for comparison between soft tissue healing in the two groups.

Time	Soft tissue healing	Laser (n = 10)		Surgery (n = 10)		*P*-value	Effect size (*v*)
		n	%	n	%		
7 days	Excellent	0	0	0	0	0.048*	0.548
	Very good	3	30	0	0		
	Good	7	70	7	70		
	Poor	0	0	3	30		
45 days	Excellent	1	10	0	0	0.087	0.529
	Very good	9	90	6	60		
	Good	0	0	4	40		
	Poor	0	0	0	0		
3 months	Excellent	5	50	2	20	0.350	0.314
	Very good	5	50	8	80		
	Good	0	0	0	0		
	Poor	0	0	0	0		

: Significant at P ≤ 0.05

Regarding the gingival marginal stability:

There was no significant difference between prevalence of gingival margin stability in the two groups when compared at baseline (immediately after surgery) and at a 3 months healing period (P=0.582) as shown in **Table (2)**.

III- Microbiological assessment:

After 7 as well as 45 days, group I showed significantly lower bacterial load than group II (P <0.001 and P=0.002 respectively) as shown in **Table (3)**. Regarding the changes within each group, both groups showed significant change in bacterial load by time. Pair-wise comparisons between time periods revealed that there was a significant decrease in bacterial load after seven days as well as from seven to 45 days (P <0.001).

TABLE (2) Frequencies (n), percentages (%) and results of Fisher's Exact test for comparison between gingival margin stability in the two groups.

Gingival margin stability	Laser (n = 10)		Surgery (n = 10)		*P*-value	Effect size (*v*)
	n	%	n	%		
Stable	7	70	9	90	0.582	0.25
Unstable	3	30	1	10		

****: Significant at P ≤ 0.05***

TABLE (3) Descriptive statistics and results of repeated measures ANOVA test for comparison between bacterial load (Copies/μl) in the two groups.

Time	Laser (n = 10)		Surgery (n = 10)		*P*-value	Effect size (*Partial Eta squared*)
	Mean	SD	Mean	SD		
Base line	1	0	1	0	Not computed	
7 days	0.63	0.07	0.82	0.06	<0.001*	0.71
45 days	0.53	0.06	0.64	0.08	0.002*	0.42

****: Significant at P ≤ 0.05***

DISCUSSION

Functional crown lengthening is a commonly used procedure to expose sound tooth structure for restoration without biologic width violation[1]. Assuming the presence of many advantages of Lasers over surgical procedure as simplicity, less bleeding, no sutures, less post-operative discomfort as well as faster healing time [11]. The current study was designed as a randomized controlled clinical trial to compare between the traditional surgical functional crown lengthening and closed flap crown lengthening utilizing Er,Cr:YSGG laser.

The Erbium laser was chosen as it has affinity for hydroxyapatite in bone as well as water in

soft tissues. So, it can be used for both soft tissue removal and bone cutting [23, 24]. To the extent of the author's knowledge, there is only one randomized controlled clinical trial that compared both methods together [18].

Soft tissue healing was assessed after 7 days, 45 days and 3 months after the procedure using the healing index of Landry, Turnbull and Howley. Group I showed statistically significant better healing after 7 days than group II while there was no statistically significant difference after 45 days and 3 months. However, group 1 showed clinically better healing after 45 days and 3 months (90 % vs 60 % very good healing after 45 days, 50 % vs 20 % excellent healing after 3 months).

The effect of laser on healing occurs through the stimulation of miscellaneous biological mechanisms. At the time of surgery, laser therapy produces an increase in blood flow that results in the recruitment of proinflammatory, anti-inflammatory and growth factors to the wound site. At the initial phase of inflammation, laser therapy can stimulate degranulation of mast cells, unleashing the inflammatory response. Thereafter, phototherapy enhances the proliferation of fibroblasts, osteoblasts and epithelial cells. It also increases protein synthesis and the release of growth factors by these cells. Altogether, these events culminate in faster clinical wound healing [27].

Regarding the gingival marginal stability, there was no statistically significant difference in the gingival margin stability between the two groups in a 3 months follow-up period. Follow-up was 3 months as previous studies showed that gingival tissues take up to 3 months after crown lengthening for healing and maturation. During these 3 months period, marginal tissue changes either by tissue recession or tissue creeping [7,8,28].

This finding does not correlate with a recent study which showed more stabilized gingival margin within the laser-operated patients. Pontoriero stated that after ostectomy and osteoplasty during crown lengthening and placing the flap in an apical position, coronal creeping of the gingival margin continues until tissue maturation. That is why immediate temporization was necessary in those studies to stop tissue relapse [15,18,29].

Gingival crevicular fluid (GCF) was collected by placing paper point with standardized dimensions just within the gingival crevice for 30 seconds. The fluid is absorbed by the paper through capillary action. Paper points for GCF collection have different advantages including being more reproducible and reliable. Using paper points of standardized size provides standardized samples which are desired by the laboratory. In addition, it is easy to apply it to individual sites with less trauma [30].

After 7 days as well as 45 days in the current study, laser group showed statistically significant lower bacterial load than surgical group. That was in agreement with a study by Gutknecht and his colleagues which used Er,Cr:YSGG to reduce microorganisms within periodontal pockets. The mean percentage reduction in bacterial load after 7 days in his study was 29.8 vs 36.6 in our study. While the mean percentage reduction after 45 days was 53.83 vs 47.4 in our study. The more reduction in bacterial load after 45 days in his study could be explained as the laser irradiation was repeated three times, each in a seven-day period [31].

The reduction in bacterial load could be related to the bactericidal effects of Er,Cr:YSGG laser on periodontal pathogens. This effect is due to high absorption of the emitted light by lipopolysaccharides needed for bacterial growth. Furthermore, Er,Cr:YSGG laser has the ability to disinfect gingival sulcus and periodontal pockets without damaging blood vessels [32,33].

The significant reduction in bacterial load may be the cause of the significant better and faster soft tissue healing in group I. Oral microflora can change normal healing process through interference with gingival keratinocytes which normally proliferate, migrate and differentiate at the wound periphery

in order to cover this area and regain normal cell morphology through re-epithelization. At the presence of high bacterial load, this process may be disrupted due to change in gene expression of keratinocytes [34].

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