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Comparision of the Microbial Count in Supragingival Plaque, Gingival Crevicular Blood and Saliva Samples Immediately after Diode Laser (970 ± 15 nm) Application in Chronic Periodontitis Patients: A Randomized Controlled Split Mouth Clinical Trial

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Abstract

Background: Periodontal disease affects 80% of the adult population in the United States. Periodontal disease are biofilm initiated inflammatory conditions with presence of pathogenic bacteria. Studies shown that laser decontamination has effect on the bacteria within the sulcus, reducing the risk of bacteremia caused from instrumentation, and to lower the microcount in aerosols created during ultrasonic instrumentation.

Aim of the study: To evaluate immediate effects of a diode laser (970 ± 15 nm) on the microbial count in Supra gingival plaque, crevicular blood and saliva samples of patients suffering from chronic periodontitis.

Materials and methods: A total of 15 subjects were recruited for the study. Each patient's mouth was divided equally into two halves which were allotted randomly to a group based on coin toss method. Quadrants in group I (test group) were subjected to diode laser debridement while those of group II (control group) were subjected to saline irrigation. Supra gingival plaque, saliva and crevicular blood samples were collected before and immediately after laser debridement and saline irrigation and subsequently subjected to microbial analysis.

Results: Clinical observations showed a significant reduction in microbial count i.e. reduction in mean CFUs(CFU/ml) in both the groups while statistically-significant reduction is seen in the test group. Test group showed significant reduction in both Supra gingival plaque samples and crevicular blood in comparison to that of saliva samples.

Conclusion: Present study concludes that diode laser (970 ± 15 nm) application has immediate effects in reducing the microbial load in supra gingival plaque, crevicular blood and saliva samples in patients with chronic periodontitis compared to control group.

Keywords: Diode laser; Supragingival plaque; Crevicular blood; Saliva; Chronic periodontitis; Microbial count

Introduction

Periodontal disease has shown to affect almost 80% of the adult population in United states [1]. The etiology of periodontal disease involves a complex interplay between bacterial pathogens and the host tissues which determines the course and extent of periodontal destruction [2]. Recent research suggests that bacteria associated with periodontal disease are associated with an increased risk of bacteremia, heart disease, diabetes, stroke, premature birth [3,4], and respiratory infection in susceptible individuals [5,6]. Even though bacteria and their products may serve as risk indicators for periodontitis, there is no single aetiology for periodontal diseases (Larry Wolff, et al.).

Complete removal of bacterial deposits and their toxins from the root surface and within the periodontal pockets is not necessarily achieved with conventional, mechanical therapy [7]. In addition, access to areas such as furcations, concavities, grooves, and distal sites of molars is limited. Although systemic and local antibiotics are occasionally administered into periodontal pockets for the purpose of disinfection, with frequent use of antibiotics there is a potential risk of producing resistant microorganisms. Therefore, development of novel techniques for scaling and root planing, as well as further improvement of currently used mechanical instruments, is required. Recently the use of lasers within the periodontal pocket has become a topic of much interest and is a promising field in periodontal therapy [8].

Laser with its various characteristics, such as ablation or vaporization, hemostasis, and sterilization effect, may serve as an adjunct or alternative to conventional, mechanical periodontal therapy [9,10]. They are also accompanied by many advantages like strong hemostatic and bactericidal effects [11-13]. Preprocedural decontamination is a laser application which is done before any instrumentation, even probing. The objectives associated with this procedure is to affect the bacteria within the sulcus, reducing the risk of bacteremia caused from instrumentation, and to lower the microcount in aerosols created during ultrasonic instrumentation [14].

Regarding clinical use of the diode laser for pocket treatment, Coluzzi recommended laser soft tissue curettage at 0.4 W in continuous wave mode after mechanical debridement of root surface, followed by irradiation at 0.6 W for hemostasis and bacterial reduction, while Gutknecht et al. [15,16], suggested the use of a diode laser at 2W in

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continuous wave mode for curettage before mechanical debridement. Thus Diode lasers are bactericidal in nature and aid in coagulation [17,18].

Thus the aim of the present study is to assess the immediate effects of diode laser (970 ± 15 nm) on microbial count in Supra gingival plaque, crevicular blood and saliva samples in patients suffering from chronic periodontitis.

Materials and Methods

Study population

The study is a randomized double blind split-mouth clinical trial, carried out at Rajarajeswari Dental College & Hospital, Bangalore, India. Study protocol was approved by the Ethical Committee of the Institute and the trial is registered under Clinical Trial Reaserch Institute (Ref/2017/12/016397). The trial was a single-centre study carried out in the Department of Periodontology. Patients were explained about the study and written consent were obtained from all the patients prior to the study.

Study design

A total of 15 patients aged 35-65 years were recruited for the study after initial screening which included oral hygiene index and Russell's periodontal index to assess the amount of debris, amount of calculus and periodontal status of the patient. Patients diagnosed with chronic periodontitis were eligible for the study. Each patient's mouth was divided equally into two halves. Each half was allotted to a group based on coin toss method. The two groups are namely: Group I (test group) and Group II (control group). Quadrants in Group I were subjected to Diode laser debridement while quadrants in Group II were subjected to Saline irrigation, without scaling and root planing in both the groups. Supra gingival plaque, crevicular blood and saline samples were collected at baseline and immediately after treatment in both the groups as shown in flow chart.

Inclusion criteria

The inclusion criteria for the study were as follows: 1) Patients should have at least 20 teeth with probing depth of ≥ 5mm, clinical attachment level ≥ 3 mm and radiographic evidence of alveolar bone loss on at least 2 teeth per quadrant excluding the third molars; 2) Patients should be systemically healthy 3) Patients should not undergo any prior periodontal treatment for the past six months.

Exclusion criteria

The exclusion criteria were as follows: 1) Patients not willing to participate in the study; 2) Patients who smoke and consume alcoholic; 3) pregnant and lactating females; and 4) patients under any medication within last six months, which may alter the periodontal status like Antimicrobial therapy, NSAIDS, Calcium channel blockers, Immunosuppressive Drugs.

Sample collection

All the procedures were carried out by single examiner. Supragingival plaque, crevicular blood and saliva samples were collected from 15 patients before and immediately after laser debridement and saline irrigation. Supragingival plaque samples were collected using sterile curette (one strok and transferred into an eppendorf tube containing PBS (Phosphate buffered saline) solution. The blood samples were collected using graduated micropipettes from the gingival sulcus and stored in eppendorf tubes containing PBS solution. Pooled saliva samples were collected by 2mL syringe and stored in eppendorf tubes containing PBS solution. All the samples were subjected to microbiological analysis. Figure 1 shows the method of collection of the samples from the patients.

Sulcular debridement

In Group I (test group), diode laser of 970 ± 15 nm wavelength and 1.2 W power was used for sulcular or pocket debridement. The fibre tip was placed subgingivally and used in a non-contact mode, for 60 seconds at each site, as shown in Figure 2. In Group II (control group), each site was irrigated with saline for 60 seconds.

Microbiological Analysis

The Supragingival plaque, crevicular blood and saliva samples collected were subjected to microbiological analysis. The mean colony forming units (CFUs/ml) of bacteria were counted by growing the colonies on sheep blood agar plates. The counts were made at the end of 2nd day after inoculation of the samples on the culture plates.

Statistical Analysis

Statistical Software Package SPSS for Windows Version 22.0 Released 2013. Armonk, NY: IBM Corp. was used to perform statistical analyses. Shapiro Wilk test indicated that the data was not following

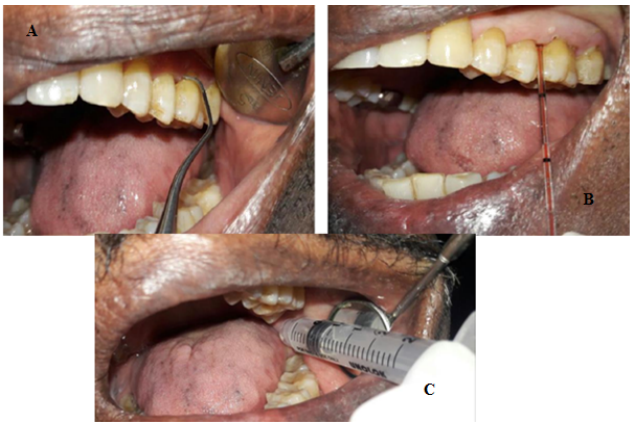

Figure 1: Method of collection of samples; (A) Supragingival plaque collection using curette (B) Crevicular blood collection using micropipettes; (C) Saliva collection using disposable syringe.

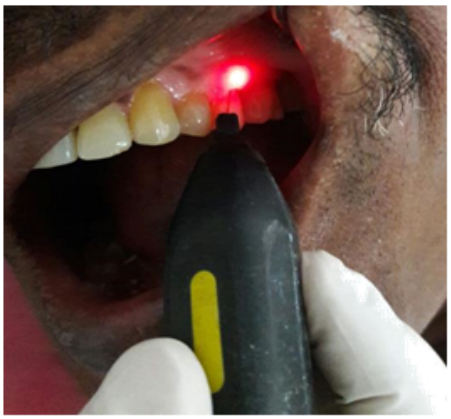

Figure 2: Diode laser debridement in non-contact mode.

a normal distribution. Henceforth, relevant non-parametric tests were used for analysis of data. Mann Whitney U test was used to compare mean CFUs between test and control group for all the samples during Pre & post treatment period. Wilcoxon signed rank test was used to compare the mean CFUs between Pre and Post treatment period in Test & Control group for the samples. Spearman's Correlation test was used to assess the relationship between the CFUs obtained from Supragingival plaque with those obtained from crevicular blood & Saliva during Pre & Post Rx period in test and control groups. The level of significance was set at $P<0.05$.

Results

Prior to debridement there was no statistically significant differences in mean colony forming unit (CFU/ml) count among the samples in both the groups as shown in Table 1 and Graph 1. Immediately after debridement both the groups showed reduction in mean CFU count but it was statistically significant in test group especially in supragingival plaque samples compared to crevicular blood and saliva samples as shown in Table 2 and Graph 2. Table 3 shows there is statistically significant reduction in mean CFU count in supragingival plaque and crevicular blood compared to saliva samples before and after debridement in test group (also shown in Graph 3). Table 4 shows the mean CFU count in all the samples in control group before and after debridement and the results revealed that there was a reduction in the mean CFU count in all the samples but it was not statistically significant (also shown in Graph 4). The results also revealed that there was a very weak positive correlation between the mean CFU values of supragingival plaque and crevicular blood and a very weak negative correlation between the mean CFU values of supragingival plaque and saliva prior to debridement, in both the groups. After debridement, the values of CFU obtained reveal that there was a weak positive correlation between supragingival plaque and crevicular blood samples and very weak negative correlation between supragingival plaque and saliva samples in test group. The correlation statistics of mean CFU between supragingival plaque, crevicular blood samples and saliva samples in the control group after debridement did not reveal statistically significant results as shown in Table 5.

Samples	Group	N	Mean	SD	Mean Diff	Mean Rank	Z	P-Value
SGP	Test	15	62200	48010.7	0	15.5	0	1
	Control	15	62200	48010.7		15.5		
Blood	Test	15	10333.3	25218.1	0	15.5	0	1
	Control	15	10333.3	25218.1		15.5		
Saliva	Test	15	58000	46475.8	0	15.5	0	1
	Control	15	58000	46475.8		15.5		

Table 1: Comparison of mean CFUs between test and control group for different types of samples during Pre-treatment period using Wilcoxon signed rank test.

Samples	Group	N	Mean	SD	Mean Diff	Mean Rank	Z	P-Value
SGP	Test	15	3220	4246.9	-	11	-	0.004*
	Control	15	49540	48982.4	46320	20	2.901	
Blood	Test	15	2413.3	3952	-7140	15.1	-	0.78
	Control	15	9553.3	25402.5		15.9	0.278	
Saliva	Test	15	46000	45638.3	-4800	15.6	-	0.94
	Control	15	50800	47728.7		15.4	0.07	

Table 2: Comparison of mean CFUs between test and control group for different types of samples during Post treatment period using Wilcoxon signed rank test.

Samples	Group	N	Mean	SD	Mean Diff	Mean Rank	Z	P-Value
SGP	Pre Rx	15	62200	48010.7	58980	8	-	0.001*
	Post Rx	15	3220	4246.9		0	3.434	
Blood	Pre Rx	15	10333.3	25218.1	7920	3.5	-	0.03*
	Post Rx	15	2413.3	3952		0	2.232	
Saliva	Pre Rx	15	58000	46475.8	12000	1.5	-	0.16
	Post Rx	15	46000	45638.3		0	1.414	

Table 3: Comparison of mean CFUs between Pre and Post treatment period in Test group for different types of samples using Wilcoxon Signed Rank test.

Samples	Group	N	Mean	SD	Mean Diff	Mean Rank	Z	P-Value
SGP	Pre Rx	15	62200	48010.7	12660	2.5	-	0.07
	Post Rx	15	49540	48982.4		0	1.841	
Blood	Pre Rx	15	10333.3	25218.1	780	2	-	0.1
	Post Rx	15	9553.3	25402.5		0	1.633	
Saliva	Pre Rx	15	58000	46475.8	7200	2	-	0.1
	Post Rx	15	50800	47728.7		0	1.633	

Table 4: Comparison of mean CFUs between Pre and Post treatment period in Control group for different types of samples using Wilcoxon Signed Rank test.

Time	Group	Sample	Values	Blood	Saliva
Pre Rx	Test	SGP	rho	0.4	-0.11
			P-Value	0.14	0.71
	Control	SGP	rho	0.4	-0.11
			P-Value	0.14	0.71
Post Rx	Test	SGP	rho	0.33	-0.09
			P-Value	0.24	0.76
	Control	SGP	rho	0.37	-0.31
			P-Value	0.17	0.26

Table 5: Spearman's Correlation test for assessing the relationship b/w the CFUs from SGP with those from Blood & Saliva.

Discussion

Chronic periodontitis is an infectious disease caused by microorganisms. The infection triggers host inflammatory responses resulting in the destruction of the tooth supporting tissues [19]. With conventional mechanical instruments, complete access and disinfection may not be achieved during the treatment of periodontal pockets. Basically, lasers have the potential advantages of bactericidal effect, detoxification effect, and removal of the epithelium lining and granulation tissue, which are desirable properties for the treatment of periodontal pockets. Some lasers may be capable of effectively removing not only dental plaque but also calculus from the root surface with extremely low mechanical stress and no formation of a smear layer on the treated root surface. Furthermore, potential biostimulation effects of scattering and penetrating lasers on the cells surrounding the target tissue during irradiation might be helpful for the reduction of inflammation and healing of periodontal tissues [20].

The diode laser basically does not interact with dental hard tissues, the laser is an excellent soft tissue surgical laser [21], indicated for cutting and coagulating gingiva and oral mucosa, and for soft tissue curettage or sulcular debridement. Some studies have demonstrated that

a diode laser facilitated bacterial elimination from periodontal pockets, resulting in better healing. Moritz et al. [22] reported pocket irradiation with a diode laser (805 nm) following scaling. Regarding clinical use of the diode laser for pocket treatment, Coluzzi [15] recommended laser soft tissue curettage at 0.4 W in continuous wave mode after mechanical debridement of root surface, followed by irradiation at 0.6 W for hemostasis and bacterial reduction, while Gutknecht et al. [16] suggested the use of a diode laser at 2 W in continuous wave mode for curettage before mechanical debridement.

In this study the immediate reduction in microbial count in supragingival plaque,crevicular blood and saliva samples was assessed after irradiation with diode laser. Our study showed laser irradiation has shown immediate reduction in microbial count in all the three samples but it was statistically significant in supragingival plaque and crevicular blood compared to saliva samples. When compared between test and control group there is statistically significant difference in reduction in microbial count in supragingival plaque samples of test group. To the best of our knowledge there are very few studies regarding the immediate effects of laser on supragingival plaque, crevicular blood and saliva samples.

Conclusion

The present study concludes that diode laser (970 ± 15 nm) application has immediate effects in reducing the microbial count in supragingival plaque, crevicular blood and saliva samples in patients with chronic periodontitis.

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