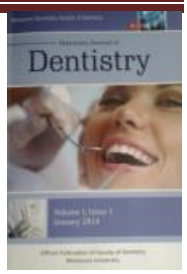

Efficacy of Locally Delivered Bifidobacterium Probiotic Gel as an Adjunctive Therapy in Periodontitis Patients (Clinical and Microbiological Study)

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Abstract:

Objectives: This study evaluates the clinical and microbiological effect of Bifidobacterium probiotic gel as an adjunctive therapy to scaling and root planning (SRP) in chronic periodontitis patients and compering this effect with Chlorhexidine gel.

Materials and Methods: Thirty chronic periodontitis patients (18 females, 12 males, aged between 30 and 50 years) were involved in the study. They were classified into 3 groups randomly. 1st group composed of 10 patients who received SRP associated with the subgingival application of Bifidobacterium probiotic gel, 2nd group composed of 10 patients who received SRP associated with the subgingival application of Chlorhexidine gel and 3rd group composed of 10 patients who received SRP only. Local drug delivery was applied once weekly for six weeks. All patients were evaluated clinically by measuring periodontal parameters (Plaque Index, Gingival Index, Probing Pocket Depth and Clinical Attachment Level) , and microbiologically by culturing plaque samples anaerobically for detection of total bacterial count, p.gingivalis & p.intermedia counts at baseline and six weeks after periodontal treatment.

Results: All periodontal parameters (PI, GI, PPD and CAL), total bacterial count in addition to p.gingivalis & p.intermedia counts were reduced significantly after six weeks among all the groups. However by comparing in between groups there was significant difference in p.gingivalis & p.intermedia counts between groups 1&3 also between groups 2& 3.

Conclusion: The local application of Bifidobacterium probiotic gel offers a promising therapeutic approach in periodontal treatment as adjunct to SRP.

Keywords: Bifidobacterium probiotic, Chlorhexidine, Chronic Periodontitis, Local Drug Delivery.

Introduction

Periodontitis is a disease of the periodontium characterized by the irreversible loss of connective tissue attachment and supporting alveolar bone. This destruction of the periodontium is a result of interactions between a complex of subgingival bacterial population and specific host defense mechanisms. (1)

Conventional periodontal treatment involves mechanical supra and subgingival debridement (Scaling and root planning) which considered as a gold standard treatment modality. (2) Although initially the number of pathogens can be greatly reduced by (SRP), periodontal pathogens quickly re-colonize the treated niches in the oral cavity. (3)

Adjunctive use of local or systemic antimicrobials improves the outcome of periodontal therapy only temporarily. Thus, a life-long need for re-treatment arises, creating a serious socio-economic problem. Additionally, increasing levels of antibiotic resistant bacteria favour the development of approaches that do not rely on antibiotics. (3)

Chlorhexidine remains the gold standard antimicrobial agents as it has a broad spectrum of antimicrobial activity that play role in treatment of gingivitis and periodontitis. Also it has an anti-inflammatory action, inhibition of bone loss and promote the attachment of fibroblast to the root surface. Moreover a great effect on plaque accumulation and bleeding on probing. (4)

Recently the use of beneficial bacteria has arisen as a promising concept in the management and treatment of periodontal diseases. (5) According to the World Health Organization, probiotics are live microorganisms that can confer health benefits to the host when consumed in adequate amounts. (6)

Probiotics have a proper scope in the field of periodontitis as they can reduce periodontopathogens, improve periodontal clinical parameters, decrease the levels of proinflammatory cytokines, and potentiate the effects of SRP. (3)

Probiotics, most commonly belong to the genera - Lactobacillus and Bifidobacterium. (6) Bifidobacterium is part of the human microbiota and presents a symbiotic relationship with the host. It is considered a potential probiotic as it possesses immunomodulatory and antimicrobial properties. (7-9)

The knowledge regarding the benefits of Bifidobacterium probiotic in the management of chronic periodontitis is limited. Further studies should be conducted to confirm the outcomes of this strain on both clinical and microbiological parameters, as well as to test new formulations of probiotics besides the common lozenges.

1. Aim of the study:

Evaluation the clinical and microbiological effect of Bifidobacterium probiotic gel as an adjunctive therapy to scaling and root planning (SRP) in chronic periodontitis patients and compering this effect with Chlorhexidine gel.

2. Patients and Methods:

3.1 patient selection:

A total of 30 patients of both genders (18 females and 12 males), aged between 30 and 50 years were selected from those attending the outpatient clinic of Oral Medicine and Periodontology department, Faculty of Dentistry, Mansoura University. They were diagnosed as having moderate chronic periodontitis according to the criteria of the International Classification System for Periodontal Diseases. Complete medical, dental histories and periodontal charting were taken from all patients. The study was approved by the internal Ethical Committee of Faculty of Dentistry, Mansoura University. All the participants clearly understood the purpose and duration of the study, expected benefits and/or complications and agreed to enroll in the study and informed consent were obtained.

3.2 Inclusion and Exclusion criteria:

The inclusion criteria were age over 30 years, probing pocket depth (PPD) ≥ 4 mm and CAL 3-4mm with no history of antibiotic or periodontal therapy in the last 3 months. The exclusion criteria were systemic conditions that could influence the progression of periodontitis or treatment response (e.g. Diabetes Mellitus), Smoking, Pregnancy, long-term administration of anti-inflammatory medications or usage of probiotics 6 months prior to the study.

3.3 Patients Grouping : The selected patients were divided into 3 groups as following:

Group I (study group): Comprised of ten patients suffering from moderate chronic periodontitis were treated by scaling and root planning (SRP) followed by local delivery of Bifidobacterium probiotic gel. **Group II (study group):** Comprised of ten patients suffering from moderate chronic periodontitis were treated by scaling and root planning (SRP) followed by local delivery of Chlorhexidine gel. **Group III (control group):** Comprised of ten patients suffering from moderate chronic periodontitis were treated by scaling and root planning (SRP) only.

3.4 Periodontal treatment:

- **Treatment phase:**

A. Full mouth scaling and root planning performed using hand instruments and ultrasonic scaler under local anesthesia when necessary for all groups.

B. Application of Bifidobacterium probiotic gel for group 1 and Chlorhexidine gel for group 2 with the same procedure by the following way: The selected sites were isolated carefully with cotton rolls and thoroughly dried then the gel was applied carefully subgingivally and interproximally until excess gel was observed from the gingival margin, excess gel was removed with a cotton roll. Patients were also instructed to avoid chewing hard or sticky foods, brushing near the gel treated site or using inter-dental aids at the day of application. Gel application was performed once a week for six weeks.

- **Bifidobacterium probiotic gel Preparation:**

Bacterial strain : Bifidobacterium bifidum, EMCC #: 1334, Designation: DSM 20082, E 319f, JCM 12, isolated from intestine of adults and supplied as actively growing cultures. It was bought from Biological Resources Center (Cairo MIRCEN), Faculty of Agriculture, Ain Shams University, P.Box 68, Hadayek Shoubra, Cairo 11241, Egypt.

The viability of the test strain was tested by monitoring their growth on Trypticase Soy Yeast Extract agar (TSYE) (Oxoid) under anerobic conditions prepared by Dr. M. Abdelmesih, (Microbilogy, F. Medicine, Mansoura University)

Gel was prepared in November 12, 2019 (Liver Research Lab-FAB-Lab, Pharmacognosy Department, Faculty of Pharmacy), Mansoura University, Egypt. Oral gel was prepared using mucoadhesive polymer to achieve acceptable mucoadhesion so that the medication remains on the spot of application for a longer time.

3.5 Clinical Assessment: Clinical parameters were recorded at the baseline and after 6 weeks of treatment including: Plaque Index (**PI**), Gingival Index (GI),Pocket Probing Depth (**PPD**) and Clinical Attachment Level (**CAL**)

3.6 Microbiological Assessment: Plaque samples were taken at the baseline and after 6 weeks of the treatment**.** Samples were cultured quantitatively on three bacterial media; first, brain heart infusion agar (Oxoid). Second, brain heart infusion agar supplemented with 5.0 ug/ml hemin for detection of Porphyromonas gingivalis. Third, Blood agar containing 10 mg sulphamethoxazole/L and 0.5 mg trimethoprim/L for detection of Prevotella intermedia. Inoculated plates were subjected to anaerobic condition using an anaerobic jar and anaerobic gas packs (Oxoid) with catalyst and then incubated at 37 C for 5 days.

3.7 Statistical analysis and data interpretation: Data were fed to the computer and analyzed using IBM SPSS Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp. Qualitative data were described using number and percent. Quantitative data were described using median (minimum and maximum) for non-parametric data and mean, standard deviation for parametric data after testing normality using Shapiro–Wilk test.

Significance of the obtained results was judged at the (0.05) level. **Qualitative data:** Monte Carlo test as

correction for Chi-Square test when more than 25% of cells have count less than 5 in tables (>2*2).

Quantitative data between groups:Parametric tests:

One Way ANOVA test was used to compare more than 2 independent groups with Post Hoc Tukey test to detect pair-wise comparison

- Paired t test to compare between 2 studied periods.

Non Parametric tests:

- Kruskal Wallis test was used to compare more than 2 independent groups with Mann Whitney U test to detect pair-wise comparison
- Wilcoxon signed Rank test to compare between 2 studied periods.

4 Results:

Regarding **Plaque Index** pre-treatment; there was no statistically significant difference between studied groups with mean Plaque index was 2.34, 2.49 & 2.33 for groups 1, 2 & 3, respectively. Post-treatment Plaque index showed statistically significant difference between groups 1&2 only. Mean Plaque index post-treatment was 0.766, 0.889 & 0.826 for groups 1, 2 & 3, respectively. Comparing pre and post treatment values of plaque index demonstrated a statistically significant lower mean values among all studied groups ($p<0.001$). Table (1)

Regarding **Gingival Index** pre-treatment; there was no statistically significant difference between studied groups with mean gingival index was 1.84, 1.815 & 1.83 for groups 1, 2 & 3, respectively. Post-treatment gingival index showed non-statistically significant difference between studied groups. Mean gingival index post-treatment was 0.356, 0.341 & 0.353 for groups 1, 2 & 3, respectively. Comparing pre-and post-treatment values of gingival index demonstrated a statistically significant lower mean values among all studied groups ($p<0.001$). Table (1)

Regarding **Pocket Probing Depth** pre-treatment; there was no statistically significant difference between studied groups with mean PPD was 3.29, 3.06& 3.28 for groups 1, 2 & 3, respectively. Post-treatment PPD showed non-statistically significant difference between studied groups .Mean PPD post-treatment was 2.02, 1.97 & 1.92 for groups 1, 2 & 3, respectively. Comparing pre-and post-treatment values of PPD illustrated a statistically significant lower mean values among all studied groups ($p<0.001$). Table (1)

Regarding **Clinical Attachment Level** pre-treatment; there was no statistically significant difference between studied groups with mean CAL was 2.79, 2.57 & 2.78 for groups 1, 2 & 3, respectively. Post-treatment CAL showed non-statistically significant difference between studied groups .Mean CAL post-treatment was 1.87, 1.96 & 2.25 for groups 1, 2 & 3, respectively. Comparing pre-and post-treatment values of CAL showed a statistically significant lower mean values among all studied groups ($p<0.001$). Table (1)

The mean value of **total bacterial count** was lowest among group 2 followed by group 1 & group 3 pre-treatment without significant difference between them with mean

bacterial count was 884 , 928.5 & 965 , respectively. Similarly , no statistically significant difference of post treatment bacterial count between studied groups with the lowest mean was detected among group 1 (505) , followed by group 2(581) and group 3(612). Table (2)

Mean values of **Prevotella intermedia count** pre-treatment had no statistically significant difference between studied groups before treatment; while there was statistically significant difference between studied groups after treatment. Mean P.intermedia count after treatment was lowest among group 1 followed by group 2 and the highest was among group 3 (11, 12 &19 respectively). Comparing pre and post treatment P.intermedia count illustrated statistically significant difference between groups (1, 3) & (2, 3) without statistically significant difference between groups (1, 2). Table (2)

Mean values of **Porphyromonas gingivalis count** pre- treatment had no statistically significant difference between studied groups before treatment. However, there was statistically significant difference between studied groups after treatment. Mean P. gingivalis count after treatment was lowest among groups 1, 2 &3 (7, 9&17 respectively) and the highest was among group 3 (17). Comparing pre and post treatment P. gingivalis count illustrated statistically significant difference between groups (1, 3) & (2, 3) without statistically significant difference between groups (1, 2). Table (2)

5 Discussion:

Periodontal diseases result from the complex interaction between pathogenic bacteria and the host immuno-inflammatory responses. [10]

Previous studies have shown that the local route of drug delivery can attain 100-fold higher concentrations of an antimicrobial agent in subgingival sites compared with a systemic drug regimen. The advantages of local drug delivery are high concentrations at the target site with reduced dosage, fewer applications, and high patient acceptability. [11]

In this connection a new approach consists of modulating the composition of the newly formed oral biofilms through the administration of probiotics concomitantly to scaling and root planning, Probiotics as living micro-organism confer health benefit on the host when administered in sufficient doses, probiotics were useful in reducing gingival inflammation and decreasing red & orange bacterial complexes count.[12]

To the best of our knowledge we were among the first to prepare Bifidobacterium probiotic in the form of gel instead of other available strains (lactobacillus) and forms (lozenges and chewing gums) as the application of gel was found to provide a long stay in the oral cavity ،possess relatively faster release and administered. Moreover, being biocompatible and allow their adhesion to the mucosa in the dental pocket. [13]

All clinical parameters were recorded at base line and 6 weeks after treatment this precise period was taken

according to ***Haffajee et al,and sokransky et al*** who proved that greatest change in probing depth reduction and clinical attachment regain occurs within 1-3 months post SRP.[14]

Regarding our results, all baseline recorded clinical parameters revealed no significant difference among studied groups because they were selected randomly to avoid bias.

Regarding **group1** treated by Bifidobacterium probiotic gel in addition to SRP; showed statistically significant improvement both clinically and microbiologically compared to the pretreatment values, this could be explained by the fact that the probiotic strain used in the present study can perfectly adhere to subgingival biofilms resulting in antimicrobial activity, as well as antioxidative and immunomodulatory properties. It can modulate the oral microbiota by directly killing or inhibition of pathogenic bacterial growth or via producing several components that act as antimicrobial agents, such as lactic acid, hydrogen peroxide and bacteriocins that may act alone or in concert in inhibiting pathogens.

In addition it works well as host-modulating agents, as they influenced the balance of proinflammatory and anti-inflammatory cytokines and reduced attachment and alveolar bone losses, the local inflammation as well favored the tissue repair. They also produce antioxidants which neutralize the free electrons which play a pivotal role in plaque formation as well as stain buildup. [15, 16]

These results came in agreement with **Invernici et al.** in their randomized clinical trial which they evaluated the effect of *Bifidobacterium animalis* subsp. *lactis* (*B. lactis*) containing probiotic lozenges as adjuvant to scaling and root planing (SRP) in patients suffering of chronic periodontitis. The patients treated with probiotic experienced superior results regarding decrease in probing pocket depth and clinical attachment gain. Furthermore, they demonstrated fewer periodontal pathogens of red and orange complexes and reduced proinflammatory cytokine levels in gingival crevicular fluid when compared with patients using placebo. [17]

Regarding **group 2** treated with Chlorhexidine gel in addition to SRP; clinically and microbiologically showed statistically significant improvement compared to their pretreatment values, this could be explained as CHX is one of the most used antimicrobial agents as it has a broad spectrum antimicrobial agents that play role in treatment of gingivitis and periodontitis. Also it has an anti-inflammatory action, inhibition of bone loss and promote the attachment of fibroblast to the root surface. Moreover a great effect on plaque accumulation and bleeding on probing. [18, 19] These results are similar to findings of several studies. [20, 21]

Our results in contrary with **Butera et al.** whom found in their study that there is no antibacterial effect of the applied probiotic and CHX pastes as well as on clinical parameters regarding CHX group .The possible explanation for the variation from that study results may be due to different concentration, forms and route of administration of applied probiotic in addition to different study design. Despite this disagreement their results found to be in accordance with our results in regard to probiotic as it has shown significance effect in improving periodontal parameters. [22]

Regarding **group3** treated by SRP only, clinically and microbiologically showed statistically significant

improvement compared to their pretreatment values. These results could be attributed to that SRP generally reduces the level of microorganisms and inflammatory markers. Also slows down the progression of periodontal disease via attachment gain and PD reduction. [23]

In this study regarding Inter-group comparison of periopathognic bacteria p. gingivals and p. intermedia count revealed marked statistical significance between probiotic and CHX groups in compression to SRP alone group.

These results explanation lie in the concept that relevant to guided pocket recolonization, (GPR) established by **Tughels et al.**, wherein the beneficial bacteria populate the sulcus and prevent the recolonization of periodontal pathogens through means of competitive inhibition by preventing the adhesion of pathogenic bacteria, competing the same nutrients. [3]

On the other hand it could be because P.gingivalis and P.intermedia were found to colonize nearly all niches in the oral cavity, such as tongue, mucosa, saliva even the tonsils. A translocation of these pathogens may occur rapidly and a recently root-planed deep pocket might be re-colonized from the remaining untreated pockets or from other intraoral niches, before a less pathogenic ecosystem can be established. [24]

Probiotics present a new ray of hope in periodontal therapy. With a proven track record of being safe and effective.

6 Conclusions:

1. Considering the clinical relevance, local application of both Bifidobacterium probiotic gel tested in this study as well as chlorhexidine gel seem to be a valid adjunctive therapy to SRP alone.
2. Bifidobacterium probiotic revealed a significant improvement on clinical indeces in addition to reduction of periopathogenic bacteria P. gingivalis and P. intermedia.
3. During this study, no side effects were detected and hence probiotic gel can be used safely in medically compromised patients.

Figure (1):
A- Case suffering from chronic periodontitis at baseline
B-Case presentation after treatment (SRP + Bifidobacterium Probiotic Gel)

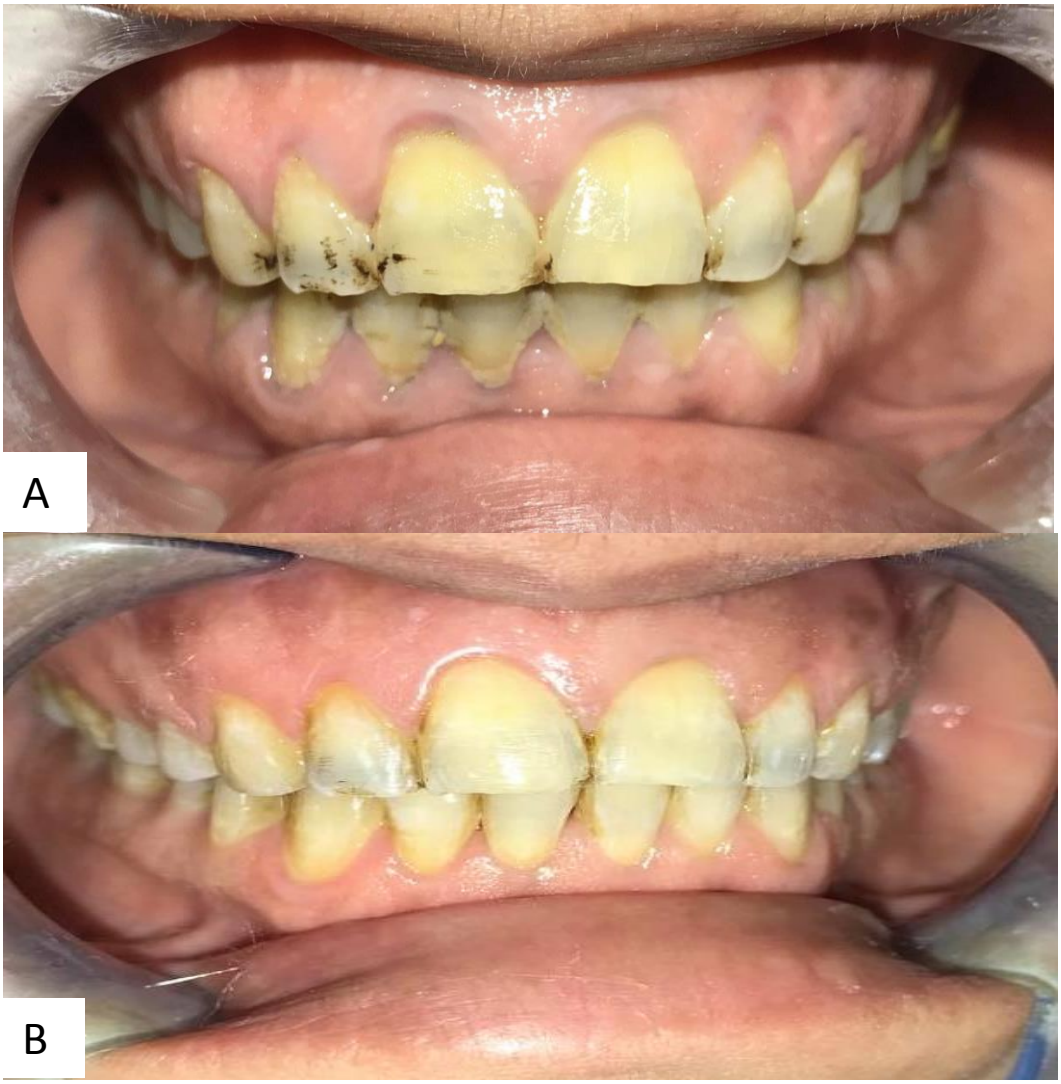

Figure (2):
C- Plaque Sampling
D- Application of Bifidobacterium Probiotic Gel)

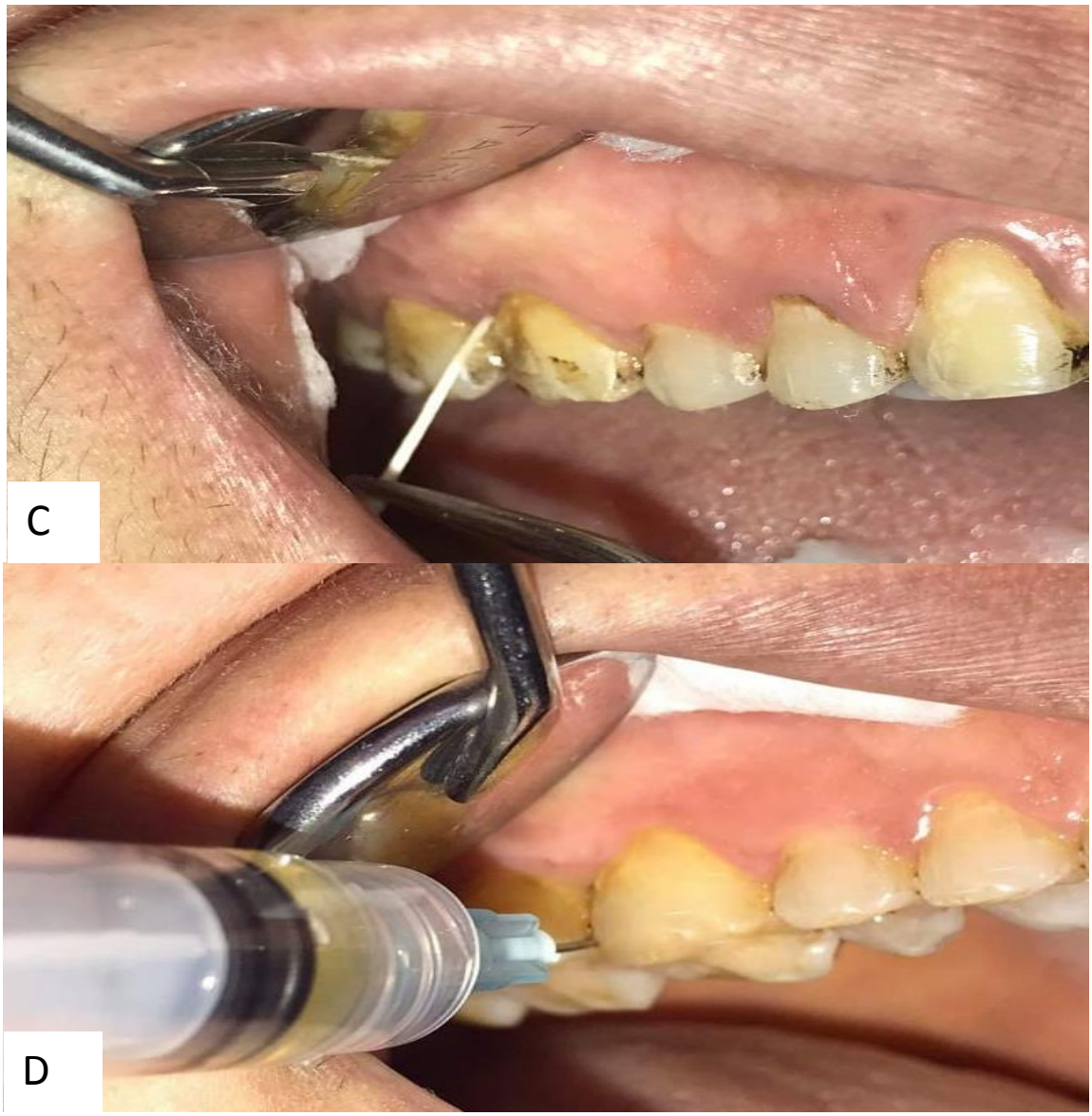

Table (1): Clinical periodontal parameters among study groups pre- and post-treatment

		Group 1 n=10	Group 2 n=10	Group 3 n=10	test of significance	within group significance
Plaque Index mean±SD	Pre	2.34±0.24	2.49±0.245	2.33±0.21	F=1.53 P=0.236	P1=0.161 P2=0.894 P3=0.127
	Post	0.766±0.096	0.889±0.138	0.826±0.109	F=2.82 P=0.08	P1=0.025* P2=0.257 P3=0.235
Paired t test p-value		<0.001*	<0.001*	<0.001*		
% of change		67.3%	62.4%	64.5%		
Gingival Index mean±SD	Pre	1.84±0.17	1.815±0.18	1.83±0.19	F=0.054 P=0.948	P1=0.749 P2=0.912 P3=0.835
	Post	0.356±0.06	0.341±0.058	0.353±0.07	F=0.147 P=0.864	P1=0.612 P2=0.919 P3=0.685
Paired t test p-value		<0.001*	<0.001*	<0.001*		
% of change		80.6%	81.2%	80.7%		
Probing depth mean±SD	Pre	3.29±0.39	3.06±0.45	3.28±0.39	F=0.969 P=0.392	P1=0.229 P2=0.958 P3=0.249
	Post	2.02±0.42	1.97±0.32	1.92±0.23	F=0.244 P=0.785	P1=0.722 P2=0.249 P3=0.737
Paired t test p-value		<0.001*	<0.001*	<0.001*		
% of change		38.6%	35.6%	41.5%		
Clinical Attachment loss mean±SD	Pre	2.79±0.34	2.57±0.34	2.78±0.31	F=1.41 P=0.26	P1=0.148 P2=0.947 P3=0.167
	Post	1.87±0.41	1.96±0.41	2.25±0.35	F=2.60 P=0.09	P1=0.609 P2=0.038* P3=0.107
Paired t test p-value		<0.001*	<0.001*	<0.001*		
% of change		32.9%	23.7%	19.1%		

Table (2): Total bacterial count + counts of P.intermedia & P. gingivalis among study groups pre- and post-treatment

		Group 1 n=10	Group 2 n=10	Group 3 n=10	test of significance	within group significance
Total bacterial count mean±SD	Pre	928.50±183.88	884.0±187.39	965.0±166.02	F=0.33 P=0.722	P1=0.584 P2=0.435 P3=0.815
	Post	505±174.42	581.0±177.60	612.0±146.35	F=1.09 P=0.35	P1=0.317 P2=0.163 P3=0.681
Paired t test		<0.001*	<0.001*	<0.001*		
P.intermedia mean ±SD	Pre	35.0±11.28	33.00±18.7	32.0±8.93	P=0.154	P1=0.775 P2=0.52 P3=0.88
	Post	11±2.5	12±1.5	19±3.1	P=0.001*	P1=0.292 P2=0.001* P3=0.001*
Paired t test		P=0.006*	P=0.001*	P=0.002*		
% of change		68.6%	63.6%	40.6%		
P.gingivalis mean ±SD	Pre	30±14.43	28±17.52	29.0±10.15	p=0.235	P1=0.197 P2=0.746 P3=0.110
	Post	7.0±1.4	9.0±2.0	17.0±1.9	p=0.002*	P1=0.644 P2=0.01* P3=0.03*
Paired t test		p<0.001*	p<.001*	p<0.001*		
% of change		76.7%	67.9%	41.4%		

F: One Way ANOVA test # by Post Hic Tukey test, p1: difference between group 1&2, p2: difference between group 1&3, p3: difference between group 2&3

KW: Kruskal Wallis test, p1: difference between group 1& 2, p2: difference between group 1&3, p3: difference between group 2&3

Table (2): Total bacterial count + counts of P.intermedia & P. gingivalis among study groups pre- and post-treatment

		Group 1 n=10	Group 2 n=10	Group 3 n=10	test of significance	within group significance
Total bacterial count mean±SD	Pre	928.50±183.88	884.0±187.39	965.0±166.02	F=0.33 P=0.722	P1=0.584 P2=0.435 P3=0.815
	Post	505±174.42	581.0±177.60	612.0±146.35	F=1.09 P=0.35	P1=0.317 P2=0.163 P3=0.681
Paired t test		<0.001*	<0.001*	<0.001*		
P.intermedia mean ±SD	Pre	35.0±11.28	33.00±18.7	32.0±8.93	P=0.154	P1=0.775 P2=0.52 P3=0.88
	Post	11±2.5	12±1.5	19±3.1	P=0.001*	P1=0.292 P2=0.001* P3=0.001*
Paired t test		P=0.006*	P=0.001*	P=0.002*		
% of change		68.6%	63.6%	40.6%		
P.gingivalis mean ±SD	Pre	30±14.43	28±17.52	29.0±10.15	p=0.235	P1=0.197 P2=0.746 P3=0.110
	Post	7.0±1.4	9.0±2.0	17.0±1.9	p=0.002*	P1=0.644 P2=0.01* P3=0.03*
Paired t test		p<0.001*	p<.001*	p<0.001*		
% of change		76.7%	67.9%	41.4%		

F: One Way ANOVA test # by Post Hic Tukey test, p1: difference between group 1&2, p2: difference between group 1&3, p3: difference between group 2&3

KW: Kruskal Wallis test, p1: difference between group 1& 2, p2: difference between group 1&3, p3: difference between group 2&3

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