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Effectiveness of probiotics as an adjunct to scaling and root planing in the treatment of chronic periodontitis – A clinical and microbiological study

[1]Dr. Saina Elahi, Senior Lecturer, Department of Periodontology and Oral Implantology, M A Rangoonwala Dental College and Hospital, Azam campus 2390-b, K.B, Hidayatulla Road, Camp, Pune, Maharashtra - 411001

[2]Dr. Salika Sheikh, Professor, Department of Periodontology and Oral Implantology, M A Rangoonwala Dental College and Hospital, Azam campus 2390-b, K.B, Hidayatulla Road, Camp, Pune, Maharashtra - 411001

[3]Dr. Sangeeta Muglikar, Professor and HOD, Department of Periodontology and Oral Implantology, M A Rangoonwala Dental College and Hospital, Azam campus 2390-b, K.B, Hidayatulla Road, Camp, Pune, Maharashtra - 411001

[4]Dr. Devika Chakravarty, Private practitioner, MDS Periodontology and oral Implantology

[5]Dr. Shibli Syed, Private practitioner, MDS Orthodontics and Dentofacial Orthopaedics

[6]Dr. Anam Khan, Private practitioner, MDS Conservative Dentistry and Endodontics

[7]Dr. Syed Shoaib, Private practitioner, MDS Conservative Dentistry and Endodontics

Corresponding Author: Dr. Saina Elahi, Senior Lecturer, Department of Periodontology and Oral Implantology, M A Rangoonwala Dental College and Hospital, Azam campus 2390-b, K.B, Hidayatulla Road, Camp, Pune, Maharashtra - 411001

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Abstract

Background: Conventional periodontal treatment such as scaling and root planing (SRP), is not effective in complete removal of all type of periodontal pathogens and their toxins. Studies have shown that the use of probiotics containing Lactobacillus species in patients with Chronic Periodontitis diminishes or decreases the number of periodontal pathogens like Aggregatibacter actinomycetemcomitans and Candida albicans. They are also believed to inhibit the growth of Porphyromonas gingivalis and Prevotella intermedia bacteria, strongly implicated in the etiology of periodontal diseases.

Aim: This study aimed to evaluate the effectiveness of probiotics containing Lactobacilli reuteri as an adjunct to scaling and root planing (SRP) in treating chronic periodontitis, clinically and microbiologically, and also to evaluate if different modes of probiotic administration

(lozenges and tonic) would have an effect on the clinical and microbiological outcomes.

Materials and method: It was a prospective clinical and microbiological study. Patients were selected based on inclusion criteria and randomly allocated into group A (SRP), group B (SRP with probiotic tonic) and group C (SRP with probiotic lozenges). Clinical outcomes like plaque index (PI), gingival index (GI), bleeding on probing (BOP), probing pocket depth (PPD), relative attachment level (RAL) and microbiological analysis in terms of colony forming units (CRUs) were evaluated at baseline 21st and 42nd day. The descriptive data was explored in terms of mean and standard deviation. The intragroup comparison was carried out by repeated measure one way ANOVA while intergroup comparison was carried out using ANOVA with Bonferroni's post hoc test. The statistical significance was kept at $p<0.05$ and the data analysis was done using SPSS software version 25.0.

Results: A total of 60 patients were included of which 33 were male and 27 were female. PI was used to monitor oral hygiene and was seen to have improved significantly across all the three groups at 21 days. However, after 42 days only the test groups improved significantly. GI was used to assess gingival inflammation. It showed a significant improvement in all groups on 21 and day 42 compared to baseline as well as between 21days and 42 days. Bleeding on Probing in all the three groups was statistically significant at both 21 and 42 days as compared to baseline as well as at 42 days. PPD showed that all three groups had statistically significant reduction in PPD on day 21 and day 42 (p-value<0.001) compared to baseline; and also on day 42 compared to day 21. A statistically significant improvement was seen in RAL at baseline compared to day 21 and day 42 (p-value<0.001) while microbiological analysis of subgingival plaque samples, showed that average CFUs of gram-negative anaerobic bacteria reduced significantly in all groups at day 21 and day 42 when compared with baseline (p-value<0.001); and in day 42 when compared with day 21 (p-value<0.001) in all the treatment groups.

Conclusion: We found that probiotics, in both lozenges and tonic form, have beneficial therapeutic effects when administered as an adjunct to SRP in the treatment of chronic periodontitis. Also, probiotics were more effective when administered in the form of lozenges as compared to tonic. Probiotics could hence be considered as an effective adjunct in treating patients with chronic periodontitis and also in patients in whom surgical therapy is contraindicated.

Keywords: Microbiology, Periodontitis, Probiotics, Root Planing, Scaling.

Introduction

Periodontitis is an inflammatory disease of the supporting tissues of the teeth caused by an array of micro-organisms resulting in either rapid or slow destruction of the periodontal tissues with pocket formation, recession or both.1 Chronic Periodontitis is the most common form of periodontitis.[1] The primary etiology of chronic periodontitis is bacteria. It has been recognized that although bacterial pathogens initiate periodontal inflammation, the host response to these pathogens is equally important in mediating connective tissue breakdown, including bone loss.[2,3] Successful periodontal management is dependent on controlling the periodontopathogens along with a microbial shift towards healthy flora, controlling tissue destruction and elimination of etiologic factors.[4]

Conventional management of periodontal disease is broadly divided into Non- Surgical Therapy and Surgical Therapy. Both these therapies target removal of the

etiological agents and aim at eliminating the micro-organisms by total elimination of plaque and calculus by mechanical debridement, leading to disruption of the subgingival biofilm and reducing the bacterial load by elimination of bacteria.[5,6]

Surgeries aid in gaining access and visibility to areas for instrumentation and in modifying hard and soft tissues to eliminate niches of plaque collection and gain maintainable areas and achieve regeneration wherever possible. Scaling and root planing (SRP) is considered as the gold standard of periodontal therapy.[6]

Since the recent past, treatment has also been focusing on modulation of the host response by suppressing the destructive aspects of host immune-inflammatory response while enhancing the protective aspects by host modulation therapy. Various potential approaches to host modulation include the use of Omega 3 fatty acids, Growth factors and use of SDD (Sub-Antimicrobial dose of Doxycycline) etc.[7]

Conventional periodontal treatment, in some cases, is not effective in complete removal of all type of periodontopathogens and their toxins. This may be due to inaccessibility of certain areas for instrumentation and maintenance or due to the tissue invasive nature of certain bacteria.[8]

Of late, a new treatment gaining popularity in the field of medicine in general, and periodontics in particular, is aimed at shifting the microflora from pathogenic towards a healthy flora. The required microbial shift towards a healthy flora can be done through the use of probiotics. Probiotics are microorganisms proven to exert health promoting influences in humans and animals.[9] They are an attractive alternative to antibiotics and target particular periodontal pathogens, thus increasing the long-term success of periodontal therapy.[10] Probiotics convert the oral flora into a host friendly biosystem by affecting the harmful bacteria by pitting them against beneficial bacteria.

Studies by Meurman JH and Stamatova I (2007 & 2009)[11], have shown that the use of probiotics containing *Lactobacillus* species in patients with Chronic Periodontitis diminishes or decreases the number of periodontal pathogens like *Aggregatibacter actinomycetemcomitans* and *Candida albicans*. Probiotics have also been shown to inhibit the growth of pathogens like *Porphyromonas gingivalis* and *Prevotella intermedia* in studies conducted by Vivekananda MR, Vandana KL, Bhat KG. (2010) and Teughels W et al (2013).[12]

Probiotics are provided in products in four basic forms- a culture concentrate added to a beverage or food; inoculated into prebiotic fibers; inoculated into a milk-based food (dairy products such as milk, milk drink, yoghurt, yoghurt drink, cheese); and, concentrated and dried cells packaged as dietary supplements (such as powder, capsule, gelatin tablets).[13] Probiotics are available in the form of lozenges, tablets, capsules, powder, mouth washes, chewing gums and tonics for general as well as dental use.

Hence, this study aimed to evaluate the effectiveness of probiotics containing Lactobacilli reuteri as an adjunct to scaling and root planing (SRP) in the treatment of chronic periodontitis and evaluate the clinical and microbiological outcomes after the treatment. At the same time, this study also aimed to evaluate if different modes of probiotic administration (lozenges and tonic) would have an effect on the clinical and microbiological outcomes.

Materials And Method

This study was a prospective, clinical and microbiological study carried out to evaluate and compare the clinical and microbiological effectiveness

of probiotics, as an adjunct to SRP, in the treatment of chronic periodontitis, after 21 days and 42 days of therapy.

60 subjects (33 male and 27 female), with chronic periodontitis were selected from the outpatient Department of Department of Periodontology and Oral Implantology. The age of the patients ranged from 36 to 58 years.

The study was approved by the Institution's Ethical committee. The nature and purpose of the study was explained to all the patients and an informed written consent was taken from all the patients. The patients were recruited in the study based on the following criteria:

A) Inclusion criteria:

1. Patients of either sex between the ages of 30-60 years.
2. Patients with untreated moderate chronic generalized periodontitis (as characterized by AAP International Workshop for classification of Periodontal diseases, 1999):

a) Pocket depth 4-6mm

b) Loss of attachment 3-4mm- present in at least 3 teeth per quadrant.

3. Systemically healthy patients with no detectable signs of any diseases or oral manifestations of any systemic diseases.
4. Teeth present ≥ 18 teeth (not including third molars and teeth with orthodontic appliances, bridges, crowns, and implants).
5. Patients who consented to be a part of the study

B) Exclusion criteria:

1. Patients consuming tobacco, in any form.
2. Patients with known systemic disease precluding to the treatment.
3. Patients who had undergone periodontal therapy 6 months prior to inclusion in the study.
4. Pregnant women and lactating mothers.
5. Patients who had taken systemic antibiotics within previous two months.
6. Patients with history of long -term use of NSAIDs within the previous year.
7. Patients with history of alcohol abuse.

Following this, the participants were randomly allotted into the following groups:

Group A (Control Group): 20 patients treated with scaling & root planing alone.

Group B: 20 patients treated with scaling & root planing + prescribed probiotic tonic (containing *Lactobacillus reuteri* [100 billion per 100 ml] with traces of *Lactobacillus casei, Lactobacillus acidophilus, Bifidobacterium bifidum*)- CytoFlora® **as shown in Figure A below**

Figure A: prescribed probiotic tonic

Group C: 20 patients treated with scaling & root planing + prescribed probiotic lozenges [containing *Lactobacilli reuteri* (200 million CFU) per lozenge]- Prodentis® **as shown in Figure B below**

Figure B: prescribed probiotic lozenges

Clinical Evaluation A detailed case history was recorded for each patient which included chief complaint, medical and dental history and a thorough intraoral examination including gingival and periodontal findings.

The following clinical parameters were recorded:

1. Plaque index (Silness and Löe, 1964)[14]
2. Gingival index (Löe and Silness, 1963)[15]
3. Bleeding on probing
4. Probing pocket depth (PPD)
5. Relative attachment level (RAL)

Measurements: Study models and customized acrylic stents16 were prepared for all the patients. Grooves were prepared in the stents with the help of no. 559 fissure bur at the site of maximum probing depth in an occluso-apical direction. Stents were prepared to standardize the probe angulation and the reference point. The same stent was used for the follow up measurements after 21 days & 42 days. PPD and RAL were measured with the help of UNC 15 probe.

Microbiologic Evaluation: Microbiological samples were collected from all the patients on their first visit. The area was isolated with sterile gauze and dried with a three way air spray to avoid contamination with saliva. Supragingival plaque, if any, was removed with a sterile curette to ensure the collection of subgingival microbial flora in the plaque sample. Subgingival plaque samples were collected from the deepest pockets with the help of paper points (No.20). After collection, the plaque samples were transported in Robertson's cooked meat medium **as shown in figure 3 below**

Figure 3: Robertson cooked meat medium to the microbiology lab within one hour of collection. Samples were labeled and processed immediately. 4.5ml of thioglycollate broth was taken in a sterile test tube to which 0.5ml of test sample was added. Serial dilutions were obtained and plated on sterile blood agar plates. This procedure was repeated for the test and control samples. The plates were then loaded in an anaerobic jar along with gas pack and indicator tablet. The anaerobic jar was incubated in an incubator at 37ºC for 24 hours. (The indicator tablet changes color to pink in the anaerobic environment.) After 24 hours, the number of colonies formed in each plate was recorded **as shown in figure 4, 5 and 6**. Aerotolerance test was performed to confirm the anaerobic nature of the colonies.

Colony forming units were calculated using the following formula:

$CFUs = y \times 10^{-d} \times 1/v$

where, d=dilution plated; v=volume plated, and y=colony count on the plates (between 30 to 300).

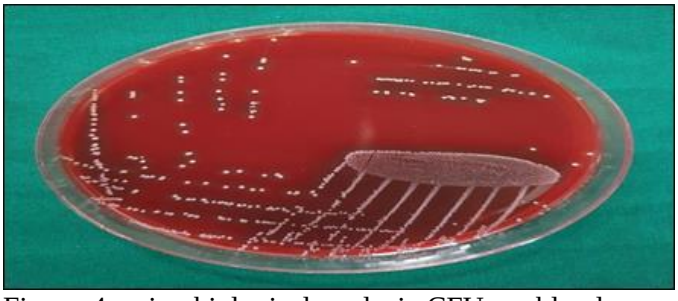

Figure 4: microbiological analysis-CFU on blood agar plates for Group A on 42nd day

Figure 5: microbiological analysis-CFU on blood agar plates for Group B on 42nd day

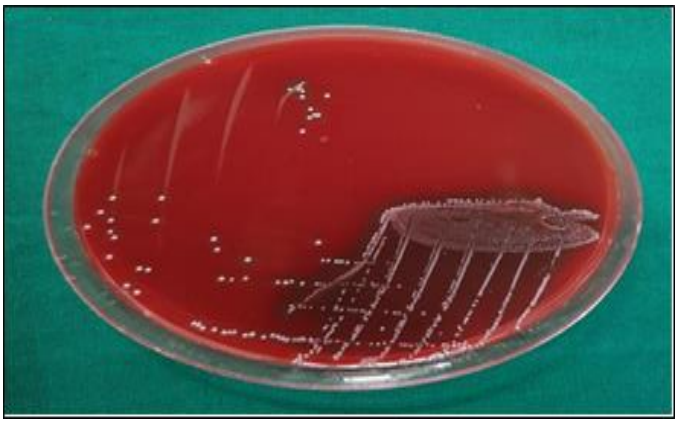

Figure 6: microbiological analysis-CFU on blood agar plates for Group C on 42nd day

Follow up measurements

The clinical parameters were rerecorded and plaque samples were collected for microbiological evaluations 21 days and 42 days after therapy. The clinical findings recorded at 21 days and 42 days included gingival index, plaque index, bleeding on probing, probing pocket depth and relative attachment level. Relative attachment level was measured using the same stent which was used at baseline. Microbiological samples were collected at 21 days and 42 days and sent for processing and analysis. Following the study period, those patients who require further periodontal treatment were advised the same.

Statistical Analysis: The data on qualitative characteristics- plaque index, gingival index, bleeding on probing, probing depth and relative clinical attachment level, is shown as n (% of cases) and the data on quantitative characteristics is presented as Mean ± Standard Error of Mean (SEM) . The entire data was entered and cleaned in MS Excel and was statistically analyzed using Statistical Package for Social Sciences (SPSS) software. The inter-group difference of quantitative variables was tested using one-way Analysis of Variance (ANOVA) with Post-Hoc Bonferroni's test. The intra-group comparisons were done using repeated measures Analysis of Variance (ANOVA) in each study group. The p-values less than 0.05 were considered to be statistically significant. The entire data was statistically analyzed using SPSS (SPSS ver 11.5, Inc. Chicago, USA) for MS Windows.

Results

A total of 60 patients were included of which 33 were male and 27 were female and the sex distribution did not differ significantly across three study groups. The mean ± standard error of mean (SEM) of age of the cases from Group A, Group B and Group C was 48.2 ± 1.41, 50.0 ± 1.25 and 49.1 ± 0.94 years respectively and the age distribution did not differ significantly across three study groups.

Clinical outcomes measured: the outcomes were measured in terms of plaque index, gingival index, bleeding on probing, pocket probing depth, relative attachment level and microbiological analysis as described below.

Plaque Index- The intragroup comparison showed that in Group A, the average baseline PI (1.82 ± 0.03) was significantly higher compared to 21 days (0.34 ± 0.03) and 42 days (0.38 ± 0.02) post treatment PI (p-value<0.001). In Group B, the average baseline PI (1.85 ± 0.04) was significantly higher compared to 21 days (0.38 ± 0.02) and 42 days (0.32 ± 0.02) post treatment PI (p-value<0.001) while in Group C, the average baseline PI (1.86 ± 0.04) was significantly higher compared to 21 days (0.36 ± 0.02) and 42 days (0.30 ± 0.02) post treatment PI (p-value<0.001) while intergroup comparison revealed that the average baseline and 21 days PI did not differ significantly across three study groups (p-value>0.05 for all). The average 42 days post treatment PI was significantly higher in group A compared to group C (p-value<0.05). The average 42 days post treatment % change in PI was significantly higher in group C compared to group A (p-value<0.05) **as shown in table 1 below.**

Table 1: The group comparison of Plaque Index across three study groups

Plaque Index	Group A (n=20)	Group B (n=20)	Group C (n=20)	Inter Group Comparisons (P-value)		
				Group A v/s Group B	Group A v/s Group C	Group B v/s Group C
Baseline	1.82 ± 0.03	1.85 ± 0.04	1.86 ± 0.04	0.999^{NS}	0.999^{NS}	0.999^{NS}
21-Days	0.34 ± 0.03	0.38 ± 0.02	0.36 ± 0.02	0.776^{NS}	0.999^{NS}	0.999^{NS}
42-Days	0.38 ± 0.02	0.32 ± 0.02	0.30 ± 0.02	0.112^{NS}	0.013^{*}	0.999^{NS}
% Change at 42-Days	79.1%	82.4%	83.4%	0.091^{NS}	0.016^{*}	0.999^{NS}

Gingival index- In Group A, the average baseline GI (1.79 ± 0.05) was significantly higher compared to 21 days (0.74 ± 0.02) and 42-day (0.47 ± 0.02) post treatment GI (p-value<0.001 for both). In Group B, the average baseline GI (1.83 ± 0.05) was significantly higher compared to 21 days (0.53 ± 0.02) and 42 days (0.33 ± 0.02) post treatment GI(p-value<0.001 for both). In Group C, the average baseline gingival index (1.81 ± 0.05) was significantly higher compared to 21 days (0.40 ± 0.02) and 42-Day (0.17 ± 0.02) post treatment GI (p-value<0.001 for both) **as shown in table 2 below.**

Table 2: The group comparison of gingival Index across three study groups

Gingival Index	Group A(n=20)	Group B (n=20)	Group C (n=20)	Inter Group Comparisons (P-value)		
				Group A v/s Group B	Group A v/s Group C	Group B v/s Group C

Baseline	1.79 ± 0.05	1.83 ± 0.05	1.81 ± 0.05	0.999^{NS}	0.999^{NS}	0.999^{NS}
21-Days	0.74 ± 0.02	0.53 ± 0.02	0.40 ± 0.02	0.001***	0.001***	0.001***
42-Days	0.47 ± 0.02	0.33 ± 0.02	0.17 ± 0.02	0.001***	0.001***	0.001***
% Change at 45-Days	73.6%	81.8%	90.7%	0.001***	0.001***	0.001***

Bleeding on probing- In Group A, the distribution of incidence of BOP at baseline was significantly higher compared to BOP at 21 days and 42 days (p-value<0.001 for both). The distribution of incidence of BOP at 21 days was significantly higher compared to BOP at 42 days (p-value<0.001). In Group B, the distribution of incidence of BOP at baseline was significantly higher compared to BOP at 21 days and 42 days (p-value<0.001 for both). The distribution of incidence of BOP at 21 days was significantly higher compared to BOP at 42 days (p-value<0.001). In Group C, the distribution of incidence of BOP at baseline was significantly higher compared to BOP at 21 days and 42 days (p-value<0.001 for both) while intergroup comparison showed the distribution of incidence of BOP at baseline did not differ significantly across three study groups (p-value>0.05 for all). The distribution of incidence of BOP 21 days post treatment was significantly higher in Group A compared to Groups B and C (p-value<0.05 for all). The distribution of incidence of BOP 21 days post treatment was significantly higher in Group B compared to Group C (p-value<0.001). The distribution of incidence of BOP 42 days post treatment was significantly higher in Group A compared to Groups B and C (p-value<0.05 for all). The distribution of incidence of BOP 42 days post treatment was significantly higher in Group B compared to Group C (p-value<0.001) **as shown in table 3 below.**

Table 3: The group comparison of bleeding on probing across three study groups

Bleeding onprobing	Group A (n=20)	Group B (n=20)	Group C (n=20)	Inter Group Comparisons (P-value)		
				Group A v/s Group B	Group A v/s Group C	Group B v/s Group C
Baseline	20 (100.0)	20 (100.0)	20 (100.0)	0.999^{NS}	0.999^{NS}	0.999^{NS}
21-Days	7 (35.0)	4 (20.0)	0	0.015*	0.001***	0.001***
42-Days	6 (30.0)	3 (15.0)	0	0.013*	0.001***	0.001***
Total	20 (100.0)	20 (100.0)	20 (100.0)			

Probing Pocket Depth- In Group A, the average baseline PPD (5.13 ± 0.11) was significantly higher compared to 21 days (2.95 ± 0.07) and 42 days (2.64 ± 0.05) post treatment PPD (p-value<0.001 for both). In Group B, the average baseline PPD (5.06 ± 0.11) was significantly higher compared to 21 days (2.69 ± 0.07) and 42-Day (2.35 ± 0.06) Post treatment PPD (p-value<0.001 for both). In Group C, the average baseline PPD (5.11 ± 0.11) was significantly higher compared to 21 days (2.57 ± 0.07) and 42 days (1.93 ± 0.06) post treatment PPD (p-value<0.001 for both). while the intergroup the average baseline PPD did not differ significantly across three study groups (p-value>0.05 for all). The average PPD at 21 days was significantly higher in Group A compared to Groups B and C (p-value<0.05 for both). The average 42 days post treatment PPD was significantly higher in group A compared to groups B and C (p-value<0.001 for both). The average 42 days post treatment PPD was significantly higher in group B compared to group C (p-value<0.001). The average 42 days post treatment % change in PPD was significantly higher in group C compared to groups A and B (p-value<0.05 for both). The average 42 day post treatment % change in PPD was significantly higher in group C compared to B (p-value<0.05) **as shown in table 4 below.**

Table 4: The group comparison of probing pocket depth across three study groups

Probing pocket dept (mm)	Group A (n=20)	Group B (n=20)	Group C (n=20)	Inter Group Comparisons (P-value)		
				Group A v/s Group B	Group A v/s Group C	Group B v/s Group C
Baseline	5.13 ± 0.11	5.06 ± 0.11	5.11 ± 0.11	0.999^{NS}	0.999^{NS}	0.999^{NS}
21-Days	2.95 ± 0.07	2.69 ± 0.07	2.57 ± 0.07	0.045^{*}	0.001^{***}	0.671^{NS}
42-Days	2.64 ± 0.05	2.35 ± 0.06	1.93 ± 0.06	0.002^{**}	0.001^{**}	0.001^{**}
% Change at 42-Days	48.2%	53.4%	61.8%	0.015^{*}	0.001^{**}	0.001^{**}

Relative attachment level- In Group A, the average baseline RAL (7.95 ± 1.04) was significantly higher compared to 21 days (5.55 ± 1.03) and 42 days (5.10 ± 1.03) post treatment RAL (p-value<0.001 for both). In Group B, the average baseline RAL (7.78 ± 1.03) was significantly higher compared to 21 days (5.50 ± 1.03) and 42-Day (5.09 ± 1.03) post treatment RAL (p-value<0.001 for both). In Group C, the average baseline RAL (8.05 ± 1.03) was significantly higher compared to 21 days (5.08 ± 1.05) and 42 days (4.20 ± 1.05) post treatment RAL (p-value<0.001 for both). In Group C, the average baseline RAL (8.05 ± 1.03) was significantly higher compared to 21 days (5.08 ± 1.05) and 42 days (4.20 ± 1.05) post treatment RAL (p-value<0.001 for both) while the inter group comparison revealed that the average baseline RAL did not differ significantly across three study groups (p-value>0.05 for all). The average RAL at 21 days was significantly higher in Groups A and B compared to Group C (p-value<0.05 for both). The average 42 days post

treatment RAL was significantly higher in groups A and B compared to group C (p-value<0.001 for both). The average 42 days post treatment % change in RAL was significantly higher in group C compared to groups A and B (p-value<0.001 for both) **as shown in table 5.**

Table 5: The group comparison of relative attachment level across three study groups

Relative attachment level (mm)	Group A (n=20)	Group B (n=20)	Group C (n=20)	Inter Group Comparisons (P-value)		
				Group Av/s Group B	Group A v/s Group C	Group B v/s Group C
Baseline	7.95 ± 1.04	7.78 ± 1.03	8.05 ± 1.03	0.712^{NS}	0.999^{NS}	0.999^{NS}
21-Days	5.55 ± 1.03	5.50 ± 1.03	5.08 ± 1.05	0.069^{NS}	0.001^{***}	0.001^{***}
42-Days	5.10 ± 1.03	5.09 ± 1.03	4.20 ± 1.05	0.999^{NS}	0.001^{**}	0.001^{**}
% Change at 42-Days	31.2%	34.0%	48.8%	0.999^{NS}	0.001^{**}	0.001^{**}

Microbiological analysis- In Group A, the average baseline (Colony Forming Units) CFUs ($5.3x10^6$ ± $0.06x10^6$) was significantly higher compared to 21 days ($4.2\ x10^6$ ± $0.07\ x10^6$) and 45-Day ($3.7\ x10^6$ ± $0.09\ x10^6$) Post treatment CFUs (p-value<0.001 for both). In Group B, the average baseline CFUs ($5.2\ x10^6$ ± $0.05\ x10^6$) was significantly higher compared to 21-Day ($3.6\ x10^6$ ± $0.09\ x10^6$) and 45 days ($2.6\ x10^6$ ± $0.08\ x10^6$) Post treatment CFUs (p-value<0.001 for both) while the intergroup comparison revealed that the average baseline CFUs did not differ significantly across three study groups (p-value>0.05 for all). The average CFUs at 21 days was significantly higher in Groups A and B compared to Group C (p-value<0.01 for both). The average CFUs at 21 days was significantly higher in Group A compared to Group B (p-value<0.001). The average 42 days post treatment CFUs was significantly higher in groups A and B compared to group C (p-value<0.001 for both). The average 42 days post treatment CFUs was significantly higher in groups A compared to group B (p-value<0.001). The average 21 days and 42 days post treatment CFUs was significantly higher in groups B compared to group C (p-value<0.001). The average 42 day post treatment % change in CFUs was significantly higher in Group C compared to Groups A and B (p-value<0.05 for all). The average 42 days post treatment % change in CFUs was significantly higher in Group B compared to Group A (p-value<0.05) as **shown in table 6.**

Table 6: The group comparison of microbiological analysis across three study groups

Colony forming units (CFU)	Group A (n=20)	Group B (n=20)	Group C (n=20)	Inter Group Comparisons (P-value)		
				Group Av/s Group B	Group A v/s Group C	Group Bv/s Group C
Baseline	$5.3x10^6 \pm 0.06x10^6$	$5.2 x10^6 \pm 0.05 x10^6$	$5.3 x10^6 \pm 0.06 x10^6$	0.999^{NS}	0.999^{NS}	0.999^{NS}
21-Days	$4.2 x10^6 \pm 0.07 x10^6$	$3.6 x10^6 \pm 0.09 x10^6$	$2.1 x10^6 \pm 0.07 x10^6$	0.010^{**}	0.001^{***}	0.001^{***}
42-Days	$3.7 x10^6 \pm 0.09 x10^6$	$2.6 x10^6 \pm 0.08 x10^6$	$10.4 x10^5 \pm 0.08 x10^5$	0.001^{**}	0.001^{**}	0.001^{**}
% Change at 42-Days	33.2%	49.8%	80.3%	0.035^{*}	0.001^{***}	0.001^{***}

Discussion

The concept regarding etiology of chronic periodontitis has been expanded to include genetic, host and environmental factors apart from microbiologic factors. As a result, we have been able to unfold many theories which explain the etio-pathogenenesis of periodontitis.16 The primary goal of periodontal therapy is to arrest the inflammatory disease process and the treatment involves mechanical removal of subgingival biofilm and establishment of a local environment and microflora compatible with periodontal health. The basic approach to treat periodontal infections has always been, and remains, the removal of supra and subgingival bacterial deposits by scaling and root planing.17 The treatment offered to periodontal patients by the clinician may be nonsurgical and/or surgical mechanical debridement. Nonsurgical mechanical periodontal treatment targets the removal of etiological agents and consists of scaling and root planing (SRP) and is the cornerstone of periodontal therapy and the first recommended approach to the control of periodontal infection.18 However, at times, conventional periodontal treatment such as SRP, is not effective in complete removal of all type of periodontal pathogens and their toxins. This has led to the evolution of various adjunctive therapeutic strategies such as local drug delivery of antiseptics and antibiotics, short term course of systemic antibiotics, host modulatory approaches, and gene therapy, in the management of periodontal diseases.19 One of such adjunctive treatments gaining popularity in the field of medicine in general, and periodontics in particular, is aimed at shifting the pathogenic microflora towards a healthy microflora. This microbial shift towards a healthy flora can be done by the administration of probiotics. Probiotics are microorganisms that exert health promoting influences in humans and animals.20 They are an attractive alternative to antibiotics, which have various adverse effects like emerging resistant strains, sensitivity, etc. Probiotics convert the oral flora into a host friendly biosystem by affecting the harmful bacteria by pitting them against beneficial bacteria.

Probiotics target particular periodontal pathogens, thus increasing the long-term success of periodontal therapy.21 Studies have shown that the use of probiotics containing Lactobacillus species in patients with Chronic Periodontitis diminishes or decreases the number of periodontal pathogens like Aggregatibacter actinomycetemcomitans and Candida albicans.22,23 They are also believed to inhibit the growth of Porphyromonas gingivalis and Prevotella intermedia bacteria24,25 strongly implicated in the etiology of periodontal diseases. Many studies have been performed in the field of general medicine to assess the effectiveness of probiotics over the years. But in the field of dentistry, especially periodontics, there is a paucity of such studies. Another problem with the previously conducted studies in the dental field are their heterogeneity; thus making it difficult to draw conclusions.10 Also, most of these studies have not compared the effectiveness of different modes of administration of probiotics. In fact, to our knowledge, none of the earlier studies have compared the effectiveness of probiotic lozenges to probiotic tonic in the treatment of chronic periodontitis. It was found in this study that all the three treatment modalities were effective in reducing PI, GI, BOP and PPD; in achieving attachment gain; and, in reducing gram negative anaerobic bacteria in subgingival plaque. Probiotic lozenges and SRP had the best outcomes, followed by probiotic tonic and SRP and last was SRP alone. Therefore, it can be concluded that, probiotics, in both lozenges and tonic form, have therapeutic benefits when administered as an adjunct to SRP in the treatment of chronic periodontitis. Also, probiotics are more effective when administered in the form of lozenges than in the form of tonic. The continued improvement in the average PI in the test groups can be due to the production of lactic acid by the homofermentative Lactobacilli. Lactic acid is a short 85 chain fatty acid, which can penetrate the bacterial membrane and acidify the cytoplasm by inhibiting the proliferation of certain pathogenic bacteria which contribute in the formation of plaque. Lactobacilli also inhibit the glycosyl-transferase enzyme by reducing the synthesis of glucans in the formation of the biofilm, therefore reducing plaque formation. These findings concur with the studies conducted by Vivekananda M.R. et al (2010)34 , Karuppaia et al (2013)29 and Vicario M et al (2013)128 which showed that probiotics have a positive effect on the reduction of PI. Ma et al (2004)156 studied the effects of live Lactobacillus reuteri on human epithelial cells in-vitro and found that Lactobacillus reuteri was able to block the Tumour Necrosis Factor α induced secretion of the proinflammatory Interleukin-8, upregulate Nerve Growth Factor, and inhibit Nuclear factor kappa B protein complex translocation to the nucleus. Twetman et al (2009)123 found that the pro-inflammatory cytokines: Interleukin-1β, Tumour Necrosis Factor α, and Interleukin-8 in gingival crevicular fluid were reduced by active probiotic treatment. These effects can be the probable cause of reduction in the bleeding on probing and inflammation in the gingiva. The more effective reduction in the CFUs by the probiotics can be explained by the actions of the probiotics on the Gram Negative Anaerobic bacteria. Apart from inhibiting the proliferation of certain microorganisms like Porphyromonas gingivalis, Streptococcus mutans104 and Prevotella intermedia105 by the production of lactic acid which leads to penetration of the bacterial membrane and acidification of cytoplasm, probiotics also produce hydrogen peroxide , which can inhibit the growth of pathogenic bacteria.106-108 Probiotics also

lead to 89 protein modification on the site of attachment of bacteria and produce bacteriocins which inhibit bacterial growth and attachment to the epithelial cells The better results of the probiotic lozenges as compared to the probiotic tonic can be explained by the more sustained local 86 action of the lozenges as compared to the tonic. The bacteria present in the lozenges are released and remain for a longer duration in the oral cavity, which increases their chances of reaching and colonizing various niches in the oral cavity including the gingival crevices and periodontal pockets; thus leading to a better local action. The probiotic tonic on the other hand stays for a shorter while in the oral cavity and has more of a systemic action compared to the lozenges. Similar results were seen in the studies conducted by Krasseet al (2006)119 , Toiviainen A et al (2015)131 and Nadkerny et al (2015)134 . It is however necessary to keep in mind that the study may have certain limitations. This study should be conducted in a larger sample size for more conclusive results. The follow up period was of a short duration, whereas longitudinal studies with minimum 1 year of follow up and larger sample size need to be carried out. It also needs to be seen how long the beneficial effect of the administration of the probiotics last following the stoppage of the supplement. Finally, the inclusion of placebos as additional groups in the study is necessary for more conclusive results as sufficient evidence exists to suggest that placebo effects can be real and measurable.

Conclusion

In conclusion, results from the current study showed that probiotics, in both lozenges and tonic form, have beneficial therapeutic effects when administered as an adjunct to SRP in the treatment of chronic periodontitis. Also, probiotics were more effective when administered in the form of lozenges as compared to tonic. Probiotics could hence be considered as an effective adjunct in treating patients with chronic periodontitis and also in patients in whom surgical therapy is contraindicated. The potential significance of the results of the current study is also in the context that the prevalence of chronic periodontitis is very high in the world population, in general, and in the Indian population, in particular. Probiotics offer a safe, effective and non-invasive option in non-surgical periodontal therapy as an adjunct.

References

1. Newman MG, Takei HH, Klokkevold PR, Carranza FA. The Carranza‘s Clinical Periodontology. 10th edition. *Saunders Company 2006.*
2. Page RC. The role of inflammatory mediators in the pathogenesis of periodontal disease. Journal of periodontal research. 1991 May 1;26(3):230-42.
3. Van Dyke TE, Serhan CN. Resolution of inflammation: a new paradigm for the pathogenesis of periodontal diseases. Journal of dental research. 2003 Feb;82(2):82- 90.
4. Lang NP, Lindhe J, editors. Clinical Periodontology and Implant Dentistry, 2 Volume Set. John Wiley & Sons; 2015 Mar 25.
5. Waerhaug J. Healing of the dento-epithelial junction following subgingival plaque control: ii: as observed on extracted teeth. Journal of Periodontology. 1978 Mar;49(3):119-34.
6. Cobb CM. Clinical significance of non-surgical periodontal therapy: an evidence-based perspective of scaling and root planing. Journal of Clinical Periodontology. 2002 May 1;29(s2):22-32.
7. Minkle Gulati VA, Govila V, Jain N. Host modulation therapy: An indispensable part of

perioceutics. Journal of Indian Society of Periodontology. 2014 May;18(3):282

8. Chatterjee A, Singh N, Saluja M. Gene therapy in periodontics. Journal of Indian Society of Periodontology. 2013 Mar;17(2):156.
9. Krayer JW, Leite RS, Kirkwood KL. Non-surgical chemotherapeutic treatment strategies for the management of periodontal diseases. Dental Clinics of North America. 2010 Jan 31;54(1):13-33.
10. Priyanka K, Sudhir KM, Reddy VC, Kumar RK, Srinivasulu G. Probiotics in Treatment and Prevention of Periodontal Diseases: A Systemic Review:2016
11. Drago L, Iemoli E, Rodighiero V, Nicola L, De Vecchi E, Piconi S. Effects of Lactobacillus salivarius LS01 (DSM 22775) treatment on adult atopic dermatitis: a randomized placebo-controlled study. International journal of immunopathology and pharmacology. 2011 Oct;24(4):1037-48
12. Iemoli E, Trabattoni D, Parisotto S, Borgonovo L, Toscano M, Rizzardini G, Clerici M, Ricci E, Fusi A, De Vecchi E, Piconi S. Probiotics reduce gut microbial translocation and improve adult atopic dermatitis. Journal of clinical gastroenterology. 2012 Oct 1;46:S33-40.
13. Foolad N, Brezinski EA, Chase EP, Armstrong AW. Effect of nutrient supplementation on atopic dermatitis in children: a systematic review of probiotics, prebiotics, formula, and fatty acids. JAMA dermatology. 2013 Mar 1;149(3):350-5.
14. Foolad N, Armstrong AW. Prebiotics and probiotics: the prevention and reduction in severity of atopic dermatitis in children. Beneficial microbes. 2014 Jan 24;5(2):151- 60.
15. Fuchs-Tarlovsky V, Marquez-Barba MF, Sriram K. Probiotics in dermatologic practice. Nutrition. 2016 Mar 31;32(3):289-95.
16. McFarland LV. Meta-analysis of probiotics for the prevention of antibiotic associated diarrhea and the treatment of Clostridium difficile disease. The American journal of gastroenterology. 2006 Apr 1;101(4):812-22.
17. Sazawal S, Hiremath G, Dhingra U, Malik P, Deb S, Black RE. Efficacy of probiotics in prevention of acute diarrhoea: a meta-analysis of masked, randomised, placebo- controlled trials. The Lancet infectious diseases. 2006 Jun 30;6(6):374-82.
18. Tung JM, Dolovich LR, Lee CH. Prevention of *Clostridium difficile* infection with *Saccharomyces boulardii*: A systematic review. Canadian Journal of Gastroenterology and Hepatology. 2009;23:817-821
19. Hempel S, Newberry SJ, Maher AR, Wang Z, Miles JN, Shanman R, Johnsen B, Shekelle PG. Probiotics for the prevention and treatment of antibiotic-associated diarrhea: a systematic review and meta-analysis. Jama. 2012 May 9;307(18):1959-69.
20. Mizock BA. Probiotics. Disease-a-Month. 2015 Jul 1;61(7):259-90.
21. Horvath A, Dziechciarz P, Szajewska H. Meta-analysis: Lactobacillus rhamnosus GG for abdominal pain-related functional gastrointestinal disorders in childhood. Alimentary pharmacology & therapeutics. 2011 Jun 1;33(12):1302-10.
22. Shen J, Zuo ZX, Mao AP. Effect of probiotics on inducing remission and maintaining therapy in ulcerative colitis, Crohn's disease, and pouchitis: meta-analysis of randomized controlled trials.

Inflammatory bowel diseases. 2013 Nov 25;20(1):21-35.

23. Szajewska H, Horvath A, Piwowarczyk A. Meta-analysis: the effects of Saccharomyces boulardii supplementation on Helicobacter pylori eradication rates and side effects during treatment. Alimentary pharmacology & therapeutics. 2010 Nov 1;32(9):1069-79.
24. AlFaleh K, Anabrees J. Probiotics for prevention of necrotizing enterocolitis in preterm infants. Evidence-Based Child Health: A Cochrane Review Journal. 2014 Sep1;9(3):584-671.
25. Ishimwe N, Daliri EB, Lee BH, Fang F, Du G. The perspective on cholesterol-lowering mechanisms of probiotics. Molecular nutrition & food research. 2015 Jan 1;59(1):94-105.