Clinical and Microbiological Investigation of the Effects of Probiotics Combined with Scaling and Root Planing in the Management of Chronic Periodontitis: A Randomized, Controlled Study

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Abstract

Aim: To assess the adjunctive effect of probiotics to scaling and root planing in the management of chronic periodontitis.

Materials and methods: Thirty systemically healthy subjects in the age range of 20 - 55 years suffering from chronic generalized periodontitis were selected and randomly assigned to a control group of patients who received scaling and root planing (SRP) alone, or a test group of patients who received SRP supplemented with probiotic administration, i.e., Bifilac lozenges. The following baseline clinical parameters were recorded at selected teeth: plaque index, gingival index, probing pocket depth and relative attachment level. Microbiological counts of *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis* and *Prevotella intermedia* were assessed in pooled subgingival plaque samples. The parameters were recorded again at 30 days, 45 days and 3 months from baseline.

Results: Statistically significant reductions were observed for plaque index, gingival index, and probing pocket depth, and a significant gain in relative attachment level in both groups. Microbiological analysis showed significant reduction for *P. gingivalis* at all recall intervals in the test group compared to controls. The intergroup comparison for differences in mean counts of *P. gingivalis* was found to be significant for the test group at 3 months ($p = 0.028$).

Conclusion: Probiotics can be considered as a potentially safe and effective adjunct to scaling and root planing in the management of chronic periodontitis.

Keywords: Probiotics, chronic periodontitis, scaling and root planing, P. gingivalis, P. intermedia, A. actinomycetemcomitans, Bifilac lozenges

Introduction

Periodontal disease results from complex interplay between the subgingival biofilm and the host immune inflammatory events that develop in the gingival and periodontal tissues in response to the challenge presented by bacteria (New-

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man *et al*., 2012). The composition of the oral microbiota is determined by a variety of synergistic interactions, such as food webs and intergeneric coaggregation, which facilitate their persistence in a dynamic environment. Likewise, interbacterial antagonism is an evolutionary mechanism that arms certain bacterial populations against elimination by other microorganisms (Essche *et al*., 2013). The summation of the antagonistic effects caused by so-called synergistic oral microbiota presents a substantial prevention of colonization by exogenous and opportunistic endogenous pathogens. Any disruption of this harmonic relationship between the host and commensal microorganisms is therefore considered an important factor for the development of oral pathologies, such as tooth decay and periodontal diseases (Essche *et al*., 2013). The current view on the etiology of plaque-associated periodontal inflammation considers three factors that determine whether disease will develop in a subject: a susceptible host, the presence of pathogenic species, and the reduction or absence of so-called beneficial bacteria (Anand *et al*., 2012).

Despite this evolution of knowledge, contemporary periodontal therapy primarily focuses on the elimination of periopathogens by nonsurgical mechanical plaque control and oral hygiene instructions (Haffajee *et al*., 2006). A close relationship between periodontitis and some microbial species has been strongly suggested, e.g., *Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Tannerella forsythia, Treponema denticola, Fusobacterium nucleatum* and *Prevotella intermedia* (Zambon, 1985; Boutaga *et al*., 2007; Lindhe *et al*., 2008). Novel strategies such as use of probiotics are emerging trends to modify pathological plaque to a biofilm of commensal organisms.

Probiotics are defined by the World Health Organization as live microorganisms which, when administered in adequate amounts, confer a health benefit on the host. Clinical benefits of probiotics have been clearly documented for different gastrointestinal disorders (e.g., lactose intolerance, viral and bacterial gastroenteritis and inflammatory bowel disease) and in immunotherapy against cancer and allergies (Mimura, 2004; McFarland, 2006). The oral cavity has been suggested as a relevant target for probiotic applications in recent years. So far oral probiotics have been evaluated primarily in the management of dental caries, halitosis and oral candidiasis (Gupta and Gupta, 2010; Gupta, 2011). Teughels *et al.* (2007) introduced a concept of guided pocket recolonization (GPR), and also concluded that after applying a mixture of beneficial bacteria following scaling and root planing there was a decrease in growth of repopulated bacteria, leading to further delay in recurrence in clinical symptoms of gingival and periodontal diseases.

After reviewing the pertinent literature regarding the use of probiotics in the management of periodontitis, this investigation was carried out to further perpetuate the role of probiotics as an adjunct to scaling and root planing in the management of chronic periodontitis.

Materials and methods

Thirty systemically healthy subjects aged 20 - 55 years suffering from chronic generalized periodontitis were selected for the study. Patients having moderate to severe chronic generalized periodontitis (≥ 3 mm clinical attachment loss involving > 30% of sites) who had not taken any antibiotics during the previous 3 months or anti-inflammatory drugs in the last month, had not participated in any clinical trial during the previous month, and those who had not undergone any surgical or non-surgical therapy 6 months prior to the study were included. Pregnant/nursing women, physically or mentally disabled patients, and those wearing orthodontic appliances or any removable prosthesis were excluded. The experiments were undertaken with the understanding and written consent of each subject and according to the principles of the Declaration of Helsinki. Sample size was estimated in consultation with an experienced statistician, based on differences in clinical parameters in previously published literature. With a standard deviation of 0.14 at a power of 85%, sample size was calculated as 10 subjects per group with confidence interval of 95%. Keeping in mind the possible dropouts, we planned to enroll 15 subjects per group. The study was reviewed and independently approved by the institutional ethical committee and State Health University. The approval no. is BFUHS/2K13/P-Th/1653.

Patients were randomly assigned (coin-toss method) to 2 groups of 15 patients each. Control group patients were subjected to scaling and root planing alone, and test group patients received scaling and root planing supplemented with probiotic administration, i.e., Bifilac lozenges (Tablets India Private Limited, Chennai). It is a commercially available probiotic preparation combined with prebiotics to enhance its action. Each tablet contains *Streptococcus faecalis* T-110 JPC -30 million CFU, *Clostridium butyricum* TO-A IHS-2 million CFU, *Bacillus mesentericus* TO-A JPC-1million CFU and *Lactobacillus sporogenes* IHS-50 million CFU.

At the first visit, a thorough patient history was recorded and a complete periodontal examination was carried out for each patient. After patient selection, the following baseline clinical parameters were recorded at selected indexed teeth of the Community Periodontal Index: plaque index (Silness and Löe, 1964), gingival index (Löe and Silness, 1963), probing pocket depth and relative attachment level. Microbiological counts of the following bacteria (CFU/mL) were assessed in a pooled subgingival plaque sample: *A. actinomycetemcomitans*, *P. gingivalis* and *P. intermedia*.

On the first visit, supragingival plaque was removed. The experimental sites were isolated with cotton rolls and dried with compressed air. Subgingival plaque samples were collected with sterile paper points. Three to four paper points were inserted into deep pockets and kept there undisturbed for at least 20 seconds, then transferred to a

vial containing reduced transport fluid (RTF) and sealed tightly to avoid contamination (Hartroth *et al.*, 1999). The patient plaque sample was transported to the laboratory for microbiological analysis by the culture method (Ananthanarayana and Paniker, 1990). Enriched and selective media were utilized for growth of selected anaerobes: blood agar and Brucella agar with hemin and vitamin K and Brucella agar with addition of kanamycin for *P. gingivalis* and *P. intermedia,* respectively, and incubation was done at 37 °C for 3-4 days in an anaerobic jar. Dentaid agar was used as selective medium for *A. actinomycetemcomitans*. Identification was confirmed by analysis of colony characteristics, Gram stain, and key biochemicals such as glucose, sucrose, cellibiose and arabinose. Anaerobic bacterial counts were observed and quantified thereafter.

Subjects were administered probiotic lozenges twice daily for 21 days in the test group. The patients were asked to suck the lozenges and instructed not to use any commercial probiotic food supplements (e.g., Yakult) during the course of the study. The follow-up visits for re-evaluation of clinical parameters and microbiological parameters were scheduled at 1 month, 45 days and 3 month intervals from the baseline visit.

Statistical analysis

The statistical analysis was carried out using Statistical Package for Social Sciences version 15.0 (SPSS Inc., Chicago, IL, USA). Mean and standard deviation for all parameters were calculated. The statistical significance of differences in independent variables for the intra-group measurements was analyzed using Student's *t*-test (two-tailed, paired). The statistical significance of intergroup differences in measurements was tested using an independent samples *t*-test. A two tailed *p*-value less than 0.05 was considered as statistically significant and a *p*-value ≤ 0.001 considered as highly significant.

Results

The study population consisted of 20 males (74.07%) and 7 females (25.93%). The age of subjects ranged from 20 - 55 years (mean 31 ± 8.07 for the control group and 33.46 ± 6.63 for the test group). Of 30 patients enrolled in the study, 27 patients (20 males and 7 females) completed the study. Two patients from the test group and one patient from the control group failed to attend the subsequent recall examinations and their data were excluded from the study. The findings of all parameters were evaluated and statistically analyzed.

In the test group, a statistically significant difference in mean plaque index was observed at 1 month, 45 days and 3 months from baseline, and between 1 month and 45 days ($p = 0.007$; *Table 1*). In the control group, a statistically non-significant difference was observed between mean plaque index at 1 month and 45 days interval, 1 month and 3 months interval and 45 days and 3 months interval (p = 0.873, 0.150 and 0.183) respectively (Table 2).

The mean reduction in gingival index in the test group from baseline to recall intervals of 1 month, 45 days and 3 months was 0.40 ± 0.63, 0.48 ± 0.69 and 0.41 ± 0.65, respectively, and statistically significant (*Table 1*). The mean reduction in gingival index at all intervals from baseline for the control group was significant as well *(Table 2)*. When the mean gingival index was compared at different observation periods, a difference that approached but did not quite achieve statistical significance was observed between the groups at 3 months ($p = 0.064$; *Table 3)*.

The mean reduction in probing pocket depth from baseline to all recall intervals was statistically significant for the test group [($p = 0.020$, 0.001 and 0.005 at 1 month, 45

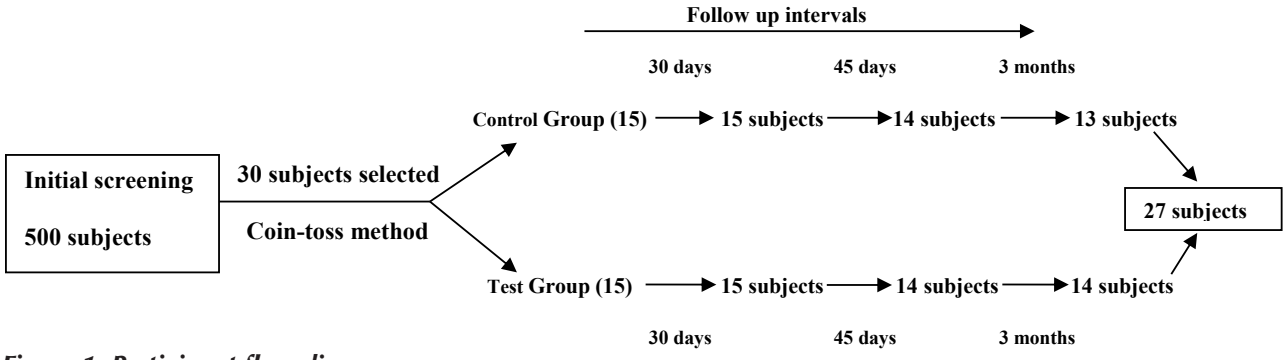

Figure 1. Participant flow diagram

Table 1. Mean values of all clinical and microbiological parameters at all periods of observation for the test group.

	Periods of observation			
Parameters	***Baseline***	***1 month***	***45 days***	***3 months***
Clinical				
PI	1.59 ± 0.34	1.29 ± 0.18	1.07 ± 0.23	1.20 ± 0.20
GI	1.90 ± 0.53	1.50 ± 0.36	1.43 ± 0.37	1.49 ± 0.35
PD (mm)	5.54 ± 1.08	4.96 ± 1.13	4.58 ± 1.05	4.62 ± 1.32
RAL (mm)	9.45 ± 1.14	8.44 ± 1.28	8.05 ± 1.36	8.16 ± 1.55
Microbiological (CFU/mL)				
***Aa* × DF**	5.82 ± 10.60	7.15 ± 24.91	13.46 ± 48.54	0.00 ± 0.00
***Pg* × DF**	21.38 ± 22.51	0.15 ± 0.38	0.54 ± 1.94	0.00 ± 0.00
***Pi* × DF**	2.00 ± 5.54	0.00 ± 0.00	0.00 ± 0.00	7.69 ± 27.74

DF, dilution factor (2×10^3); PI, plaque index; GI, gingival index; PD, probing depth; RAL, relative attachment level; *Aa, Aggregatibacter actinomycetemcomitans; Pg, Porphyromonas gingivalis; Pi, Prevotella intermedia*

Table 2. Mean values (mean ± SD) of all clinical and microbiological parameters at all periods of observation for the control group.

	Periods of observation			
Parameters	***Baseline***	***1 month***	***45 days***	***3 months***
Clinical				
PI	1.51 ± 0.40	1.24 ± 0.38	1.23 ± 0.32	1.11 ± 0.15
GI	1.81 ± 0.54	1.37 ± 0.38	1.35 ± 0.29	1.26 ± 0.26
PD (mm)	4.97 ± 0.61	4.55 ± 0.80	4.41 ± 0.72	4.15 ± 0.73
RAL (mm)	9.17 ± 0.75	8.56 ± 1.21	8.36 ± 1.07	8.21 ± 1.08
Microbiological (CFU/mL)				
***Aa* × DF**	10.57 ± 16.15	3.29 ± 12.29	5.00 ± 14.01	11.07 ± 24.03
***Pg* × DF**	28.29 ± 30.68	13.79 ± 45.28	7.00 ± 11.73	14.36 ± 21.68
***Pi* × DF**	0.71 ± 1.86	0.57 ± 2.14	0.57 ± 2.14	2.57 ± 8.05

DF, dilution factor (2×10^3); PI, plaque index; GI, gingival index; PD, probing depth; RAL, relative attachment level; *Aa, Aggregatibacter actinomycetemcomitans; Pg, Porphyromonas gingivalis; Pi, Prevotella intermedia*

Table 3. Showing comparative analysis of mean values of clinical parameters in test and control groups at different periods of observation.

Clinical parameters	***Period of observation***	***Test group***	***Control group***	***p-value***
PI	Baseline	1.59 ± 0.34	1.51 ± 0.40	0.582
	1 month	1.29 ± 0.18	1.24 ± 0.38	0.688
	45 days	1.07 ± 0.23	1.23 ± 0.32	0.166
	3 months	1.20 ± 0.20	1.11 ± 0.15	0.233
GI	Baseline	1.90 ± 0.53	1.81 ± 0.54	0.636
	1 month	1.50 ± 0.36	1.37 ± 0.38	0.358
	45 days	1.43 ± 0.37	1.35 ± 0.29	0.566
	3 months	1.49 ± 0.35	1.26 ± 0.26	0.064
PD	Baseline	5.54 ± 1.08	4.97 ± 0.61	0.102
	1 month	4.96 ± 1.13	4.55 ± 0.80	0.284
	45 days	4.58 ± 1.05	4.41 ± 0.72	0.615
	3 months	4.62 ± 1.32	4.15 ± 0.73	0.260
RAL	Baseline	9.45 ± 1.14	9.17 ± 0.75	0.464
	1 month	8.44 ± 1.28	8.56 ± 1.21	0.800
	45 days	8.05 ± 1.36	8.36 ± 1.07	0.522
	3 months	8.16 ± 1.55	8.21 ± 1.08	0.932

PI, plaque index; GI, gingival index; PD, probing depth; RAL, relative attachment level

days and 3 months, respectively (*Table 1*)] and the control group as well (*Table 2*). When mean pocket depth was compared at different observation periods, statistically non-significant differences were observed between the groups at all intervals (*Table 3*).

The mean reduction in relative attachment level for the test group from baseline to 1 month, 45 days and 3 months was 1.01 ± 0.92 mm, 1.39 ± 0.88 mm and 1.29 ± 0.70 mm, respectively, which was statistically significant (*Table 1*). When both groups were compared for mean relative attachment levels at different observation periods, statistically non-significant differences were observed between the groups at all intervals (*Table 3*).

When the groups were compared for microbiological analysis, a statistically significant difference was observed at 3 months for mean counts of *P. gingivalis*. In the test group, the mean change in bacterial counts of *P. gingivalis* from baseline to 1 month, 45 days and 3 months was statistically significant ($p = 0.005, 0.007, 0.005$) compared to the control group (*Table 1, 2*). When the groups were compared for mean counts at different observation periods, a statistically significant difference was observed at 3 months ($p = 0.028$; *Table 4*). The mean bacterial counts of *A. actinomycetemcomitans* in the test group at baseline were $5.82\times(2\times10^3) \pm 10.60$ CFU/mL and increased non-significantly to $7.15\times(2\times10^3) \pm 24.90$ CFU/mL and $13.46\times(2\times10^3) \pm 48.54$ CFU/mL at 1 month and 45 days interval with final non-significant reduction to almost non-detectable counts at 3 months follow up (*Table 1*). In test group, the mean bacterial counts of P. intermedia at baseline were 2.00 × (2 × 103) ± 5.54 CFU/ml and changed non-significantly at all recall intervals (Table 1, 2). When the groups were compared for mean counts at different observation periods, a statistically non-significant difference was observed at all recall intervals *(Table 4)*.

None of the subjects in the test group reported any adverse effects due to treatment procedure as evaluated subjectively.

Discussion

After completion of scaling and root planing, patients were administered probiotic lozenges twice daily for 21 days in the test group. The follow-up visits for re-evaluation of clinical and microbiological parameters were scheduled at 1 month, 45 days and 3-month intervals from the baseline visit. The duration of investigation was based on previous literature suggesting that initial healing should be assessed four to six weeks after performing root planing (Greenstein, 2000). Also, the major changes in clinical parameters have been reported to occur during the initial 1 - 3 months after completion of non-surgical periodontal treatment.

The results elucidated a statistically significant reduction in the mean plaque index in both groups at all recall intervals compared to baseline. However, comparison between the two groups for the mean plaque index scores revealed a non-significant difference. Decrease in plaque index scores in both groups can be explained by reduction of local deposits after mechanical debridement. In the test group, an additional effect can be attributed to the inhibition of pathogen adhesion, colonization and biofilm formation by probiotic bacteria (Gupta, 2011). Also, probiotics could modify the protein composition of the pellicle by binding and degradation of salivary proteins and lower the pH, thus making microorganisms unable to adhere for biofilm formation (Karuppaiah *et al.*, 2013). Further, the results may be affected by the so-called Hawthorne effect, as a response to subject motivation at the beginning of the study and the anticipation of forthcoming oral examination at intervals during the study. Results are quite in agreement with various studies on different probiotic supplements

Table 4. Showing comparative analysis of mean values (CFU/mL) of all microbiological parameters in test and control groups at different periods of observation.

Parameters	*Period of observation*	*Test group*	*Control group*	*p-value*
Aa	Baseline	5.82 ± 10.60	10.57 ± 16.15	0.385
	1 month	7.15 ± 24.91	3.29 ± 12.29	0.619
	45 days	13.46 ± 48.54	5.00 ± 14.01	0.555
	3 months	0.00 ± 0.00	11.07 ± 24.03	0.108
Pg	Baseline	21.38 ± 22.51	28.29 ± 30.68	0.510
	1 month	0.15 ± 0.38	13.79 ± 45.28	0.280
	45 days	0.54 ± 1.94	7.00 ± 11.73	0.062
	3 months	0.00 ± 0.00	14.36 ± 21.68	0.028*
Pi	Baseline	2.00 ± 5.54	0.71 ± 1.86	0.439
	1 month	0.00 ± 0.00	0.57 ± 2.14	0.336
	45 days	0.00 ± 0.00	0.57 ± 2.14	0.336
	3 months	7.69 ± 27.74	2.57 ± 8.05	0.532

*$p < 0.05$; DF, dilution factor (2×10^3); *Aa*, *Aggregatibacter actinomycetemcomitans*; *Pg*, *Porphyromonas gingivalis*; *Pi*, *Prevotella intermedia*

(Krasse *et al*., 2006; Shimauchi *et al*., 2008; Vivekananda *et al*., 2010; Jain *et al*., 2011; Dhawan and Dhawan, 2013; Karuppaiah *et al*., 2013; Toiviainen *et al*., 2014). Increase in plaque index scores at the end of the study period in the test group could possibly be related to lack of patient compliance for so long and the indefinite status of persistence of probiotic bacteria in the oral cavity after discontinuation of probiotic lozenges.

The mean gingival index scores at all periods of observation compared to the baseline were significantly reduced in both groups. The intergroup differences in mean gingival index scores were statistically almost approached significance at 3 months. The reduction in gingival index scores by mechanical therapy is due to removal of plaque and other local deposits contributing to its accumulation, whereas in the test group, the role of probiotics in reducing production of proinflammatory cytokines through their immunomodulatory actions on nuclear factor-κB pathways, increasing production of anti-inflammatory cytokines such as IL-10 as evidenced by previous literature, should be considered. *P. gingivalis*, being a "red complex" bacteria, is strongly correlated with disease activity and severity. *Lactobacilli* has been documented to strongly inhibit *A. actinomycetemcomitans, P. gingivalis* and *P. intermedia.* Inhibition of *P. gingivalis* by lactobacilli in the test product could be possibly associated with the reduced gingival inflammation. Results are in accordance with the findings of several other studies (Krasse *et al*., 2006; Shimauchi *et al*., 2008; Harini *et al*., 2010; Jain *et al*., 2011; Vivekananda *et al*., 2010; Dhawan and Dhawan, 2013; Teughels *et al*., 2013; Toivianen *et al*., 2014). An observation in the test group at the end of the study period was a rise in the gingival index, which differed significantly from that in the control group. This can possibly be related to the parallel rise in *P. intermedia* counts in the test group at the end of the study period. It is well known that the re-emergence of periodontopathogens is correlated with the risk for disease relapse (Teughels *et al*., 2007).

A statistically significant reduction in mean pocket depth in both groups at all recall intervals compared to baseline was observed. This can be explicated on the basis of reduction in inflammation after removal of local factors by scaling and root planing. However, in the test group, the additional role of probiotics through various underlying mechanisms viz. killing or inhibition of growth of pathogens through production of bacteriocins or other products, inhibition of collagenases and reduction of inflammation associated molecules, modulation of the host immune response, modulation of cell proliferation and apoptosis, should also be considered. Similar results have also been reported by Teughels (2007, 2013). Other investigations by Vivekananda *et al.* (2010) and Tsubura *et al.* (2012) revealed a rise in mean pocket probing depth at 30 days. These differences in findings can be explained on the basis of a multitude of underlying factors, e.g., different study methods, test product, insufficient oral hygiene, and questionable retention period of probiotic bacteria. Analyzing the different follow-up times of various studies in previous literature, it seems that the use of probiotic lozenges results in more drastic pocket depth reductions during the early healing periods (Teughels *et al*., 2007).

A similar trend of observations as in probing pocket depth changes were seen for relative attachment level, i.e., significant gain of attachment in both groups at all recall intervals compared to baseline with non-significant differences when compared against each other. The gain in attachment post-mechanical debridement could be explained by gain in new attachment, healing by long junctional epithelium or change in soft tissues, and in the test group, inhibition of collagenases and reduction of inflammation-associated molecules by probiotics may also be acknowledged. Moreover, a rise in recorded values in all clinical parameters in the test group at the last follow-up visit points towards limited sustainability of the effects of probiotic supplementation in the oral cavity. The results for the current investigation are in concordance with those reported by Vivekananda *et al.* (2010) and Teughels *et al.* (2013).

When microbiological parameters were assessed, a significant difference in reduction of mean counts of *P. gingivalis* was observed for the test group compared to the control group at the end of the study, and a significant decrease in mean counts was seen in the test group at all periods of observation compared to the control group. Results are similar to those demonstrated by Tsubura *et al.* (2009), Vivekananda *et al.* (2010) and Iniesta *et al.* (2012). Probiotics play an important role in oral ecology by specifically preventing the adherence of other bacteria and by modifying the protein composition of salivary pellicle. The reduction can be explained by microbial antagonism of the *Lactobacilli* strain in the test product toward *P. gingivalis,* substantially more than *A. actinomycetemcomitans* and *P. intermedia,* as also reported by Essche *et al.* (2013), who suggested that the growth inhibition caused by the lactic acid bacteria toward the pathogens was mainly caused by the production of large amounts of organic acids, because the neutralization of supernatants pH completely eliminated the antagonistic activity. This is especially true in case of *P. gingivalis*, which stops growing if the environmental pH drops below 6.5. Current investigation observed most significant alterations in the counts of *P. gingivalis* even compared to other test organisms. This has been already substantiated by Teughels (2013) and is of particular clinical significance, as *P. gingivalis* is considered as a keystone pathogen that can create dysbiosis between the host and dental plaque.

A non-significant reduction was observed in mean

counts of *A. actinomycetemcomitans* by the end of the study compared to baseline in the test group. An overall fluctuating trend in mean counts was observed in test group with final reduction to minimal counts at the end of the study period. It seems that the effect of probiotics became evident over a longer period of time. At initial recalls, mean counts were comparable in the two groups before finally rising in the control group. It appeared that a single session of scaling and root planing was capable of disturbing the proportions of certain bacterial forms in the subgingival periodontal flora, but the effects could not be sustained over a longer duration, with proportions returning to baseline levels at approximately 42 days (Mousques *et al.*, 1980). Similar results have been demonstrated by Vivekananda *et al.* (2010), Iniesta *et al.* (2012) and Teughels *et al.* (2013).

For *P. intermedia,* mean counts were observed to decrease for 45 days and subsequently rose at 3 months in both groups non-significantly. It has been observed by Essche *et al.* (2013) that *P. intermedia* is not substantially inhibited compared to *P. gingivalis* and *A. actinomycetemcomitans* by *Lactobacilli* strains. Results are similar to earlier studies by Vivekananda *et al.* (2010) and Iniesta *et al.* (2012), who reported significant reduction in mean counts at 42 days and 8 weeks respectively, whereas Teughels *et al.* (2013) reported significant reduction at 3 months too. The results do not suggest that a permanent installation can take place in persons with established microflora. In fact, a concordant trend in clinical parameters is also observed at the end of the study in the test group. Also, differences in composition of commercial probiotic preparations, i.e., selected strains used in the aforesaid investigations, different dosage prescription, sample population, and length of evaluation period could possibly affect the outcome.

So far, this was the first investigation evaluating the effect of Bifilac lozenges on clinical and microbiological parameters in periodontal disease, so direct comparison with previous literature was not feasible. Earlier Narayanappa (2008) and (Dhawan and Dhawan, 2013) evaluated the efficacy of Bifilac in diarrhea and gingivitis respectively and reported no adverse effects associated with their usage.

The findings of the present investigation elucidated significant reduction of *P. gingivalis* and greater reduction in clinical parameters in subjects receiving probiotics and scaling and root planing as compared to the subjects receiving scaling and root planing only, as evident from their mean differences, although the differences between groups were not of the magnitude to be statistically significant. With this short evaluation time, the reported microbial shifts probably did not have sufficient time to induce any significant clinical effect in all parameters as previously evidenced by Iniesta *et al.* (2012) and Teughels *et al.* (2013).

Conclusion

Within the limitations of the study, probiotics (Bifilac lozenges) can be considered as an effective adjunctive therapy to scaling and root planing in the treatment of chronic periodontitis.

Because periodontal disease is essentially a disease with microbial etiology, in conditions altering the oral ecology towards pathogenic shift, e.g., smoking, diabetes, hormonal fluctuations, or refractory disease, probiotics may provide an additional layer to modulate the ecologic patterns. So the need of the hour is more strictly designed, controlled and follow-up trials to explore their real potential in management of diseases. Probiotics, being a novel therapy, need further exploration in terms of strain specificity for different disease conditions and safety issues. Future research and longitudinal studies with larger sample size along with concomitant biochemical and novel microbiological analysis are recommended to further ascertain these findings.

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