The evaluation of probiotic as an adjunct to scaling and root planing in chronic periodontitis patients - A clinical, microbiological and biochemical study

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Abstract

Introduction: As probiotics are emerging as an essential treatment modality for chronic periodontitis its role is not well established. This study aimed to evaluate the efficacy of probiotic as an adjunct to Scaling and Root Planing (SRP) in chronic periodontitis. The clinical parameters, the levels of Matrix Metalloproteinases 8 in gingival crevicular fluid and the presence & reduction of Porphyromonas gingivalis in subgingival plaque samples were determined.

Materials and Methods: This clinical study was conducted for 6 weeks with a single evaluator to investigate the effect of probiotic as an adjunct to SRP in chronic periodontitis. The clinical parameters were analyzed at baseline, 3 weeks and 6 weeks. This study also evaluated the effect of Probiotic on Matrix metalloproteinase 8 levels in GCF and on the existence of Porphyromonas gingivalis in subgingival plaque after 3 weeks.

Results: Clinical parameters, gingival crevicular fluid Matrix Metalloproteinase 8 and Subgingival plaque levels of Porphyromonas gingivalis were reduced in Control (Group I) and Probiotic study (Group II) at baseline and 6 weeks. On comparing the groups, Group II had a significant decrease in clinical, microbiological and biochemical parameters than Group I, post treatment.

Conclusion: Probiotics have great potential in arena of periodontics in terms of plaque formation, altering anaerobic bacterial colonization, reduction in Pocket Depth, gingival bleeding and clinical attachment level and in treating halitosis. Probiotics can be used as an effective adjunctive therapy to conventional non-surgical periodontal treatment in Chronic Periodontitis patients.

Keywords: Chronic periodontitis, Probiotics, Scaling and root planing, Gingival bleeding, Halitosis.

Introduction

Chronic periodontitis is a bacterially induced complex inflammatory disease that destroys the connective tissue and alveolar bone, in which the disease expression involves interaction of the pathogens with host immune inflammatory response which leads to subsequent alteration in homeostasis.[1] Teeth with chronic periodontitis usually has very complex deposits of polymicrobial communities on affected root surface. The extracellular matrix degradation in chronic periodontitis is due to the presence of virulence factors and colonization of host tissue by Porphyromonas gingivalis, which is the main causative organism of periodontal diseases.[2] There is strong evidence that A.actinomycetescomitans and tannerella forsythia are periodontal pathogens, the main causative organism for chronic periodontitis. There are moderate evidence to support an etiological role for campylobacter rectus, eubacteriumnodatum, prevotella intermedia, nigrescens, parvimonasmicra, and the streptococcus intermedius complex and treponemadenticola. The periodontal treatment rationally involves nonsurgical debridement of plaque and calculus. Root planning is a debridement process defined for removal of dental plaque and calculus and also for smoothening of exposed root surfaces thereby removing dentine or cementum that is impregnanted with toxins, calculus and microorganisms.[3] Along with nonsurgical periodontal therapy, antibiotics are used as an effective adjunctive in treating periodontal disease. Anti infective treatment is very effective in arresting the progression of chronic periodontitis and it supports the host defense system in overcoming the subgingival microbial colonization. But the most severe complication of anti-infective therapy is the development of bacterial resistance.[4] In order to overcome the emergence of antibiotic resistance and frequent recolonization of pathogenic bacteria in treated sites, there was a need for a new paradigm and it was fulfilled by the advent of probiotics in the field of periodontics.[5] As per the latest evidences, probiotics play a role in oral ecology.

Probiotics are living organisms which when administered in adequate amounts, has beneficial health effects on host. Probiotics gets easily adapted to host as they are body's own resident flora and they are free from concerns of developing resistance.[6] Probiotics have antibacterial effect which is exercised by inhibiting pathogen adhesion, colonization and biofilm formation thereby producing bacteriocins for killing or inhibiting the growth of periodontal pathogens.[7] Probiotics inhibit the Matrix Metalloproteinases and inflammation associated molecules. Probiotics also act by inducing the expression of cytoprotective proteins and modulates the host immune system, cell proliferation and apoptosis.[8]

The present study investigates the effect of Probiotic (BIFILAC) as an adjunct to scaling and root planing in chronic periodontitis. This study also evaluates the effect of probiotic (BIFILAC) on Clinical Parameters, Matrix Metalloproteinase 8 levels in Gingival Crevicular Fluid and on the presence of Porphyromonas gingivalis in subgingival plaque post treatment.

Materials and Methods

A single blind randomized controlled, prospective clinical study was conducted for six weeks with 60 participants assigned to test and control group using lottery method. Patients with mild to moderate chronic periodontitis, as defined by probing depth ≥ 4 mm to ≤ 7mm and generalized interproximal attachment loss were considered in the study. Both males and females in the age group of 25-50 years who attended the outpatient department of Periodontics, J.K.K. Nattraja Dental college and Hospital, Komarapalayam were included in the study. The Institutional Ethical clearance was obtained and it was monitored in accordance with Declaration of Helsinki and Good Clinical Practice (ICH-GCP) guidelines. Smokers and alcoholics, pregnant and lactating women, patients treated with antibiotics in the past 6 months, participants allergic to lactose and fermented milk products and to other drugs, patients with systemic illness and HIV infection/AIDS were excluded from the study. Prior to initiating the study, the patients were informed of the purpose and design of the study and were requested to sign an informed consent. The control group (n = 30) received standard therapy and placebo, whereas the study group (n = 30) received standard therapy along with Probiotics. Complete medical/dental history, clinical and oral examination were done. Site specific scoring was followed, where in, the site showing most severe inflammatory signs or greater amount of attachment loss was selected for GCF and plaque sample collection. The clinical parameters were analyzed at baseline, 3 weeks and 6 weeks. The Biochemical and Microbiological parameters were evaluated at baseline and 3 weeks. Dosage of Probiotic lozenges was decided based on the previous researches and the clinical features of the study population.[9] Test group received SRP along with Probiotic lozenges twice daily for 3 weeks, whereas the control group received SRP along with Placebo lozenges twice daily for 3 weeks.

Scaling and Root planing involves removal of dental plaque and calculus, along with cementum or dentine that is impregnated with calculus, toxins, or microorganisms. At baseline full mouth ultrasonic scaling was done in the test and control sites. Under local anesthesia with 2% lignocaine solution (1:80,000), root planning was done in the test and control sites using area-specific double-ended Gracey curettes (Hu-Friedy).

The sites were isolated by cotton rolls and gently air-dried to remove saliva and a 2 µL sterile glass micro capillary tube was placed at the opening of the periodontal pocket for collecting GCF. Sterile Gracey curette were introduced into the specific site and the plaque sample from the subgingival site is obtained. Both GCF and subgingival plaque samples were placed in separate sterile, labeled Eppendorf tubes containing 500 µL of sterilized PBS buffer and samples were immediately frozen at -20ºC for further processing. The clinical assessment parameters were Plaque Index, Gingival Index, Sulcus Bleeding Index, Probing Pocket Depth, clinical Attachment Level. The Biochemical parameter was to evaluate the levels of Matrix Metalloproteinase 8 and the Microbiological parameters was to assess the presence of Porphyromonas gingivalis.

The study product (BIFILAC-lozenges) contains Lactobacillus sporogenes 100 million, Streptococcus faecalis T-110 JPC 60 million, Clostridium butyrium TO-A 4 million and Bacillus mesentericus TO-A JPC 2 million. Combination of probiotic strains has been used that acts synergistically and enhances the possibility for permanent installation. Participants were instructed to place the lozenges in the oral cavity for a few minutes as it has the benefit of increased bioavailability and site specificity. The results obtained were analyzed statistically and comparisons were made within each group using students paired ‘t test’ and ‘p value’ between baseline, 3 weeks and 6 weeks post-treatment were evaluated. The statistical analysis was done using SPSS software Version 19.

Results

Total of 60 patients selected for the study were divided into 2 groups, Group I (SRP +Placebo) and Group II (SRP+ Probiotic). No adverse reaction was observed in any subject, and no patient reported any discomfort. All subjects tolerated the drug, without any complications. At baseline, the mean Gingival Index score was 2.07 ± 0.40 in Group I and 2.09 ± 0.53 in Group II, which reduced to 1.84 ± 0.47 and 1.12 ± 0.42 at the end of 6 weeks in Group I and II respectively with P value < 0.001. In the same period, the mean Plaque Index score was reduced from 2.12 ± 0.51 to 1.92 ± 0.43 and from 2.15 ± 0.48 to 1.04 ± 0.51 in Group I and Group II respectively having P value < 0.001. There was significant improvement in Sulcus Bleeding Index at baseline and 6 weeks post-therapy in both the groups from 2.30 ± 0.47 to 1.96 ± 0.43 in Group I and from 2.24 ± 0.42 to 1.13 ± 0.49 in Group II with P value < 0.001 as shown in Table 1. At end of six weeks, the mean probing pocket depth was reduced from 5.19 ± 0.14 mm to 1.69 ± 0.57 mm in group II, whereas in Group I it reduced from 5.13 ± 0.10mm to 2.50 ± 0.54 mm. Clinically, the probing pocket depth reduction was more evident in Group II compared to Group I after 6 weeks as shown in Fig. 1 and 2. The mean Clinical Attachment Level at baseline of both the groups was 3.00 ± 00 mm. In group I the CAL was 1.27 ± 0.38 mm and in group II it was 0.54 ± 0.47 mm after 6 weeks as shown in Table 2. The percentage reduction in probing depth was 51.7% and 77.4% in Group I and II with P value < 0.05. The percentage gain in CAL in Group I was 56.7% and in Group II 84.4% having a P value of < 0.05. At baseline, the mean level of Matrix Metalloproteinase 8 in Group I was 40.7 ± 0.61 ng/mL and Group II was 40.9 ± 0.36 ng/mL which was reduced to 32.3 ± 0.38 ng/mL and 20.2 ± 0.67 ng/mL in Group I and II respectively having a P value < 0.05 as shown in Table 3. In evaluating the presence of Porphyromonas gingivalis, more positive bands of P gingivalis was seen in both Group I and Group II at baseline and there was much reduction after 3 weeks in Group II after post therapy compared to Group I as shown in Fig. 3.

Table 1: Comparison of Indices between Group I and Group II

Index	Group I		Group II		P Value
	Baseline	6 Weeks	Baseline	6 Weeks	
Gingival Index	2.07 ±0.48	1.84 ± 0.47	2.09± 0.53	1.12 ± 0.42	< 0.001*
Plaque Index	2.12 ± 0.51	1.92 ± 0.43	2.15 ± 0.48	1.04 ± 0.51	< 0.001*
Sulcus Bleeding Index	2.30 ± 0.47	1.96 ± 0.43	2.24 ± 0.42	1.13 ± 0.49	< 0.001*

Table 2: Comparison of reduction in PPD and gain in CAL between Group I and Group II

Clinical Parameter	Group I		Group II		P Value
	Baseline	6 Weeks	Baseline	6 Weeks	
Probing Pocket Depth	5.13 ± 0.10	2.50 ± 0.54	5.19 ± 0.14	1.69 ± 0.57	< 0.001*
Clinical Attachment Level	3.09 ± 0.21	1.27 ± 0.38	3.05 ± 0.13	0.50 ± 0.47	< 0.001*

Table 3: Comparison of MMP 8 levels in GCF between Group I and Group II using ELISA

MMP 8 Levels in GCF	Group I	Group II	p – value
Baseline	40.7 ± 0.61	40.9 ± 0.36	-
3 Weeks	32.3 ± 0.38	20.2 ± 0.67	< 0.05*

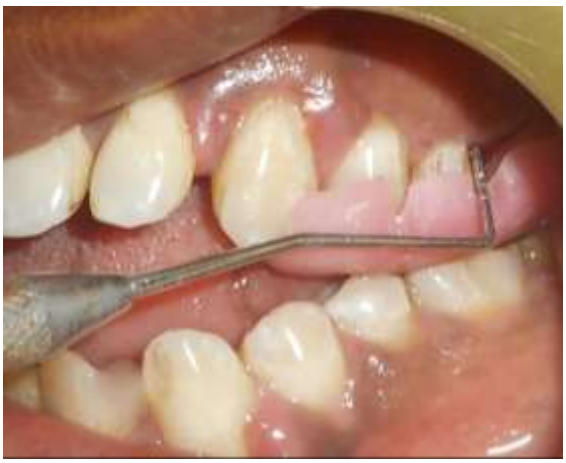

Fig. 1: Placebo group

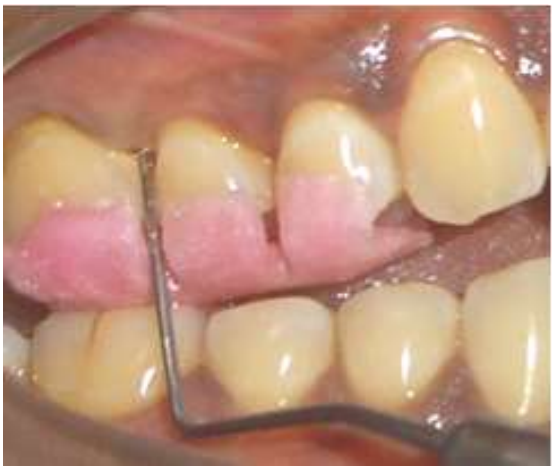

Fig. 2: Probiotic group

Figure 1 and 2, shows reduction in probing depth after 6 weeks of SRP in Group I and II.

Probing pocket depth reduction was more significant in Probiotic group compared to placebo group.

Group I and Group II using Conventional PCR

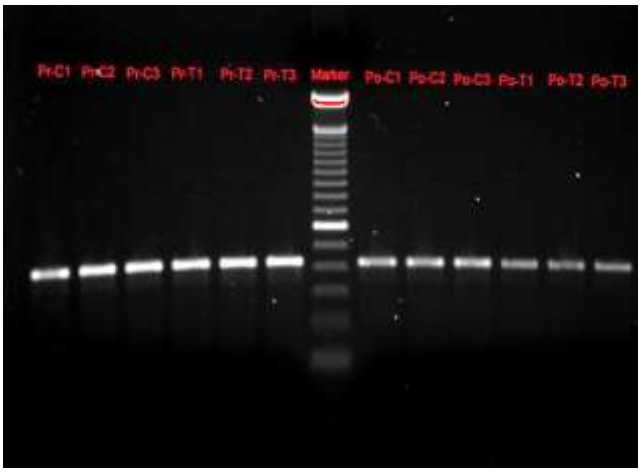

Fig. 3: Comparison of presence of porphyromonas gingivalis between

Discussion

Periodontal diseases are a group of diseases characterized by inflammation of periodontium and the subsequent destruction of the tooth supporting tissue. Chronic periodontitis is a disease that progresses slowly and generally becomes clinically significant in adults. Probiotics uses harmless bacteria to displace pathogenic organisms and it is a promising way of combating infections. WHO and FAO of the United States in 2001 defined probiotics as living microorganisms which when administered in adequate

amounts confer a health benefit on this host. Probiotics in the oral cavity, lowers the pH so that the periodontopathogens cannot form plaque and calculus, which forms the primary etiological factor.[10]

Scaling and root planning accompanied by oral hygiene procedures have served as a gold standard of periodontal therapy. Scaling and root planing done in both the groups and subjects in Group I are given Placebo lozenges and Group II are given Probiotic lozenges twice daily for 3 weeks. There was significant reduction in Clinical parameters at 3 weeks and 6 weeks post treatment in both the groups. This was in accordance with the study done by Litty et al 2015[11] in his study used probiotic containing Streptococcus salivarius M18 in adjunct to SRP and found that there was reduction in clinical parameters at 30 and 60 days and he also stated that probiotic inhibits plaque formation by lowering the salivary pH, production of antioxidants and by neutralizing the free electrons which are needed for the mineralization of plaque.[11]

In this study, the Probing Pocket Depth reduced to 51.7% in Group I and 77.4% in Group II, after 6 weeks. Similarly, in Group I there is 56.7% gain in Clinical Attachment Level and in Group II it was 84.4% after 6 weeks of treatment, which suggests a statistically significant gain in Clinical Attachment Level in Group II. This was supported by Teughels et al 2013 [12] in which the study proved the significant reduction in Clinical parameters such as decrease in Probing Pocket Depth and increase in Clinical Attachment Level gain compared to placebo group.

The evaluation of MMP 8 levels in GCF reveals that, the mean post-operative MMP 8 levels in Group I was 32.3 ng/µl and Group II was 20.2 ng/µl suggesting more reduction of MMP 8 levels in Probiotic group compared to Placebo group. This is in accordance with a study by Ince G et al (2015)[13] who explained that there was significant decrease in levels of Matrix metalloproteinase 8 and increase in TIMP level in Test group treated with probiotic than the control group. Haukioja A (2010)[14] stated that probiotics enhances the host immune response by inhibiting the production of pro inflammatory cytokines and MMPs and prevents the progression of the inflammatory disease process. The anti-inflammatory effects of Probiotic (L. brevis) could be attributed in preventing the production of Nitric oxide and consequently decreases the release of Prostaglandin E2 and Matrix Metalloproteinases.[15]

The presence of Porphyromonas gingivalis after 3 weeks of probiotic therapy is also evaluated and the results showed a marked reduction in the levels of Porphyromonas gingivalis in Group II compared to Group I. The study done by Vivekananda et al (2010)[16] supported that the microbial levels of Aggregatibacter actinomycetemcomitans, Porphromonas gingivalis, Prevotella intermedia were significantly reduced in patients who consumed probiotic (Lactobacillus reuteri). The inhibitory activity displaced lactobacilli against periodontal pathogens was principally related to their production of acid and not due to H_2O_2. The probiotic bacteria competes for the adhesion sites, thereby reduces the growth factors and nutrients available for the pathogens to sustain in the oral cavity.

Conclusion

The applications of health promoting bacteria for therapeutic purposes in general and oral health is one of the strongest emerging field and at present they are supplied as dietary probiotics for health benefits. Clinically available data show that probiotics heal to improve the oral health.

Based on the results obtained from the present study, the probiotic (BIFILAC) showed significant changes in Clinical parameters, along with the decrease in levels of inflammatory mediator Matrix Metalloproteinase 8 and marked reduction in levels of Porphyromonas gingivalis when used as an adjunct to Scaling and Root Planing.

Limitation of the study includes the small sample size and short follow-up duration. Further long term studies have to be conducted to understand the ability of probiotic bacteria to survive, grow, and to find out the best possible probiotic strains, means of their administration in different oral health conditions to assess its clinical significance.

Conflict of Interest: None.

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