Cumhuriyet Dental Journal, 26(3): 220-226, 2023
DOI: https://doi.org/10.7126/cumudj.1091906

Cumhuriyet Dental Journal

| cdj.cumhuriyet.edu.tr | Founded: 1998 | Available online, ISSN: 1302-5805 e-ISSN: 2146-2852 | Publisher: Sivas Cumhuriyet Üniversitesi

Probiotic Chewing Gums for Adjuctive Treatment of Periodontitis in Diabetics

Kalyanam Rashmi Durga[1,a*], Ravindra S.[1,b], Prakash M Halami[2,c]

[1] *Department of Periodontology, JSS Dental college and Hospital, Mysore, Karnataka*
[2] *Department of Microbiology and Fermentation Technology, CSIR-CFTRI, Mysore, Karnataka*
**Corresponding author*

Research Article

History

Received: 03/04/2022
Accepted: 02/07/2023

ABSTRACT

Introduction: The treatment of periodontal disease in diabetic subjects should also focus on lowering blood glucose levels, which might act as an adjuvant to conventional periodontal treatment. In the form of probiotics, bacterial therapy offers a dual role in controlling blood glycemic levels and reducing colonization of oral bacteria.
Aim: To evaluate the efficacy of probiotics in managing periodontitis among diabetic and non-diabetic subjects.
Methodology*:* This study was designed as a randomized, double-blinded clinical trial among diabetic and non-diabetic subjects with periodontitis. Twenty-four subjects in each diabetic and non-diabetic group were randomly assigned into two probiotic test sub-groups and one placebo sub-group. *Lactobacillus fermentum* MCC2760 and *Bifidobacterium longum* NCIM5684 probiotic chewing gums were provided to subjects in test groups to use twice a day for 30 days. Supragingival plaque samples were collected at baseline and 30 days to analyze total bacterial count and subgingival plaque for *P.gingivalis, A.actinomycetemcomitans* through quantitative polymerase chain reaction(qPCR). Clinical parameters were recorded at baseline, 30, 45, and 90 days.
Results: After 30 days, a significant reduction in plaque index, gingival Index, probing pocket depth, and gingival bleeding index was observed in scaling and root planing group(SRP) and SRP+probiotic groups. There was a significant reduction in total bacterial count among probiotic groups compared to placebo. qPCR analysis revealed non-significant reduction of *p.gingivalis* and *A.actinomycetemcomitans* in test groups. Intergroup comparison between diabetic and non-diabetic groups did not show any significant differences either in clinical or microbial parameters.
Conclusions*:* probiotic functional foods can be delivered as an adjunct to SRP to manage periodontitis in systemically compromised subjects. Long-time use of probiotics is recommended to maintain the recolonization of bacteria in periodontal tissues.

Key words*:* Probiotics, Periodontitis, Lactobacillus fermentum, Bifidobacterium longum, Aggregatibacter actinomycetemcomitans.

[a] rashmikalyanam10@gmail.com https://orcid.org/0000-0002-7946-3002
[b] adrsravindra@yahoo.co.in https://orcid.org/0000-0002-5511-9098
[c] prakashalami@cftri.res.in https://orcid.org/0000-0002-0293-1891

How to Cite: Rashmi Durga K, SR, Halami PM. (2023) Probiotic Chewing Gums for Adjuctive Treatment of Periodontitis in Diabetics, Cumhuriyet Dental Journal, 26(3):220-226.

Introduction

The prevalence of periodontal disease in India is high (51%) as half of the adults suffer from some form of periodontal disease.[1] Recent epidemiology studies have stated that periodontitis does not follow a linear progression and is not age-dependent. In the 2018 EFP/AAP case definition, a participant was a periodontitis case if: interdental CAL ≥ 2 non-adjacent teeth, or Buccal or Oral CAL ≥ 3 mm with PPD > 3 mm is detectable at ≥2 teeth.[2] However, its initiation and progression are strongly influenced by host susceptibility, local and systemic risk factors.[3]

Diabetes mellitus and periodontitis are polygenic disorders with some grade of immuno-regulatory dysfunction.[4] Diagnostic criteria by the American Diabetes Association (ADA) for type 2 diabetes include the following:[5]

- A fasting plasma glucose(FPG) level of 126 mg/dL or higher, *or*
- A 2-hour plasma glucose level of 200 mg/dL or higher during a 75-g oral glucose tolerance test (OGTT), *or*
- A random plasma glucose of 200 mg/dL or higher in a patient with classic symptoms of hyperglycemia or hyperglycemic crisis, *or*
- A hemoglobin A1c (HbA1c) level of 6.5% or higher

There is emerging evidence that supports the existence of a two-way relationship between diabetes and periodontitis. Diabetes increases the risk for periodontitis, and periodontal inflammation negatively affects glycemic control.[6] However, the mechanism that underpins the link between these conditions is limited to the aspects of immune functioning, neutrophil activity, and cytokine biology.[6]

Scaling and root planning(SRP), the conventional treatment for periodontitis, will not entirely eliminate the pathogenic bacteria as they may reside at sites inaccessible for instrumentation. So, mechanical therapy combined with antibiotics or antibiotic combinations

offered satisfactory results as they significantly suppressed the growth of periodontal pathogens. But, in the recent era, antimicrobial resistance(AMR) has been the current global issue due to its overuse and misuse. Drug resistance of bacterial dental biofilm has unlatched newer approaches to non-surgical periodontal therapy(NSPT). In this process, probiotics evolved as a trending bacteria as they might provide an opportunity for replacement therapy in bacterial-mediated oral diseases.[7]

The FAO/WHO defines probiotics as "live microorganisms which, when administered in adequate amounts, confer a health benefit on the host."[8] probiotics combat infections by displacing pathogenic bacteria and replacing them with harmless beneficial microorganisms. Research highlighted the success of probiotics in many areas of medicine, such as treating gastrointestinal tract and oropharyngeal infections.[9] The immunomodulatory and anti-inflammatory properties of probiotics help treat periodontitis. Probiotics also reported having a favorable impact on the metabolic control of subjects with type 2 diabetes.[10]

Lactobacillus and Bifidobacterium are the most commonly used probiotic strains. Current probiotic delivery systems include mouth rinses, probiotic drops, lozenges, and dentifrice. They are also available as "functional food," which, apart from their nutritional value, apparently improves the health and well-being of consumers.[11]

In the present study, chewing gums of two different probiotic strains were delivered as an adjuvant to SRP to diabetic and non-diabetic subjects with periodontitis to assess their potency on clinical and microbial parameters.

This study aimed to evaluate the efficacy of probiotics in managing periodontitis among diabetic and non-diabetic subjects.

Material and Methods

This randomized, double-blinded (patients and examiner blinded), placebo-controlled trial was approved by the Institutional Ethics committee, JSS Dental College and Hospital (44/2019). Systemically healthy and diabetic subjects with periodontitis fulfilling the inclusion criteria were divided into three sub-groups. GROUP; A. periodontitis subjects with diabetes(A1, A2, A3) GROUP; B. periodontitis subjects without diabetes(B1, B2, B3). Subjects were allotted to *L.f* test groups(A2, B2), *B.l* test groups(A3, B3), and control groups(A1, B1) based on a Computer-generated random allocation sequence. Informed consent was taken from all the patients. This trial was registered at clinical trials.gov as CTRI/2020/10/028466.

Inclusion criteria; Age; 35 years-75 years, periodontal pocket depth ≥ 5mm, Type II diabetic subjects with glycated hemoglobin range between 7% –10 %.

Exclusion criteria; Smokers, pregnant and lactating mothers, people with a compromised immune system, antibiotic therapy during the previous six months, systemic diseases other than diabetes, subjects taking medications that could interfere with gingival tissue responses.

Plaque Index (Silness & Loe-1964), Gingival Index (Loe & Silness-1963), Probing Pocket Depth, Gingival Bleeding Index (Ainamo & Bay-1975) were recorded. Supragingival plaque samples from the buccal surface of anterior maxillary teeth and subgingival plaque samples from the pocket sites were collected by using sterile curettes. After sample collection, scaling and root planning were performed for all the subjects. In the diabetic and non-diabetic groups, subjects in the placebo subgroup were given plain chewing gums, and subjects in test subgroups were given *Lactobacillus fermentum* MCC2760 and *Bifidobacterium longum* NCIM5684 probiotic chewing gums. Each chewing gum contains $1*10^8$ CFU of probiotic bacteria. They were asked to chew it for 10 minutes, twice a day, morning 1 hour after breakfast, and at night, 1-hour post-dinner for 30 days. These chewing gums were prepared freshly every week to maintain the viability of probiotic cells. They were packed, coded, and distributed to the appropriate groups regularly once a week. Supra and subgingival plaque samples were collected after 30 days. Clinical parameters were recorded after 30, 45, and 90 days. Subjects were evaluated, and oral hygiene instructions were reinforced at each visit.

Preparation of probiotic culture & chewing gum

This probiotic culture was developed at Central Food Technological Research Institute (CSIR-CFTRI) Mysuru. The probiotic strains were activated and then passaged twice in MRS broth (pH.4 for *Lactobacillus* and pH.5 for *Bifidobacterium*). They were incubated at 37 °C for 24 hours. The strains were centrifuged at 15,000 rpm for 5 minutes, and then the supernatant was discarded. The obtained biomass of the strains was washed with 0.1M phosphate-buffered saline(PBS). The cells were suspended in PBS, and optical density was adjusted to correspond to colony-forming units per milliliter (CFU/mL). The culture was lyophilized by centrifugal methods to obtain freeze-dried cells. All chemicals were supplied by HiMedia Pvt.Ldt., India.

The chewing gum base was placed for softening in the oven for 5 hours at 50 – 60° C. In the softened gum base, sodium alginate and pectin were added in batches and blended adequately for 4 to 5 minutes. Essence was also added to the preparation. The temperature was cooled to 37° C, and freeze-dry probiotic powder was added, stirred, and allowed to cool. After cooling, they were molded into required shapes and packed. 1 gram of freeze-dried powder contains $1*10^8$ CFU. Freeze-dried powder was added based on the number of chewing gums required.

Microbiological Parameters

In the course of initiation and progression of periodontal disease, the subgingival bacteria multiply in numbers and invade the cells of pocket epithelium and underlying tissues. *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans* can invade the

gingival tissues and cause severe chronic periodontitis and aggressive periodontitis.

The supragingival plaque was analyzed for the total bacterial count and subgingival plaque for *P.gingivalis* and *A.actinomycetemcomitans* through quantitative PCR. The collected samples were transferred to the laboratory (Faculty of Life sciences, JSSAHER) in 2 hours. For total bacterial count, the pore plate technique was used. For qPCR, DNA extraction was done according to the modified method by Wilson *et al*. PCR amplification was performed in a total reaction mixture volume of 25 µl. The sequence of primers and probes for *P.g* and *A.a*[12] are

F: GCGCTCAACGTTCAGCC,

R: CACGAATTCCGCCTGC,

6FAMCACTGAACTCAAGCCCGGCAGTTTCAA-TAMRA

F: GAACCTTACCTACTCTTGACATCCGAA,

R: TGCAGCACCTGTCTCAAAGC

6FAM-AGAACTCAGAGATGGGTTTGTGCCTTAGGG-TAMRA

The samples were subjected to an initial amplification cycle of 50°C for 2 min

and 95°C for 10 min, followed by 45 cycles at 95°C for 15 s and 60°C for 1 min.

The data were analyzed with ABI 7000 Sequence Detection System software.

Statistical Analysis

The sample size was calculated based on hypothesis testing between the two means using nMaster software. The sample size was computed to be 7 per group at an assumed mean difference of 0.45 with 5% alpha error and 80% power and an effect size of 1.67. However, the sample size was rounded off to 8 per group anticipating a 10% dropout.

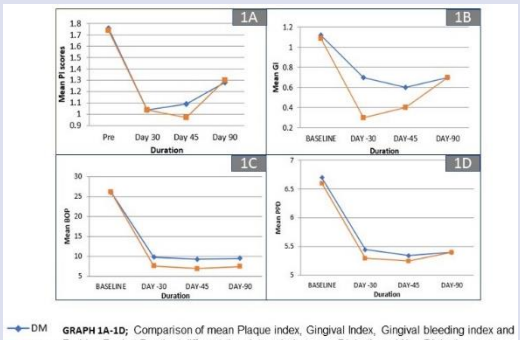
All the clinical and microbiological parameters were analyzed using SPPS version 21 software (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp). Descriptive statistics like mean and standard deviation were applied. Repeated measures ANOVA was used to know the significance in a mean difference of groups versus sessions for PI, GI, Bleeding Index, PPD, and bacterial count. Unpaired 't-test was applied for intragroup comparison at different time intervals. Statistical significance was set at $p<0.05$

Results

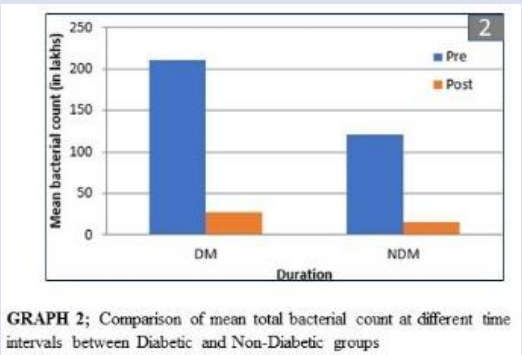
48 subjects were included in the study, and only 40 subjects were considered for final analysis, 18 females and 22 males. The mean age group of subjects in the diabetic group is 50.0 ± 11.75, and the non-diabetic group is 43.4 ± 11.75. There is no statistically significant difference between groups($F= 2.905$, $p= 0.097$) and subgroups($F= .227$, $p= 0.798$) with respect to age of subjects.

Intergroup comparison for PI, GI, Gingival Bleeding Index, PPD, and total bacterial count, from baseline to days 30, 45, and 90 showed a non-significant difference between diabetic and non-diabetic groups(graphs 1&2). *P.g* and *A.a* are slightly higher in the diabetic group, which is non-significant (graph-3 &4).

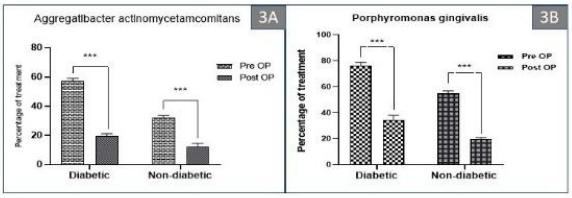
On intra-group comparison, a statistically significant reduction was observed for PI, GI, Gingival Bleeding Index, and PPD from baseline to day 30 for test and control sub-groups of both diabetic and non-diabetic groups(Tables 1-4). The total bacterial count was significantly reduced in probiotic groups(table-5). Non-significant reduction of *P.g* and *A.a* was observed in all sub-groups after 30 days (Table-6).



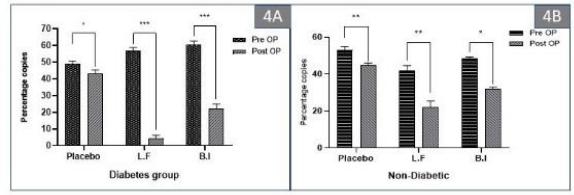
GRAPH 1A-1D; Comparison of mean Plaque index, Gingival Index, Gingival bleeding index and Probing Pocket Depth at different time intervals between Diabetic and Non-Diabetic groups



GRAPH 2; Comparison of mean total bacterial count at different time intervals between Diabetic and Non-Diabetic groups



GRAPH-3A & 3B; Comparison of *A.a* and *P.g* at baseline and 30 days between Diabetic and Non-Diabetic groups



GRAPH-4A & 4B; Intragroup comparison of *A.a+P.g* at baseline and 30 days in Diabetic and non-diabetic groups.

Table 1. Intragroup comparison of plaque index from baseline to 90 days.

Plaque İndex		Diabetic Group			Non-Diabetic Group		
		Paired diff		.sig	Paired diff		.sig
		Mean	S.D		Mean	SD	
Placebo	BL -DAY 30	0.64	0.23	0.000	0.64500	0.22474	0.001
	BL -DAY 45	0.54	0.3	0.003	0.69500	0.21843	0.001
	BL -DAY 90	0.35	0.26	0.012	0.36167	0.30195	0.032
L.f	B.L -DAY 30	0.70333	0.44189	0.011	0.79857	0.32231	0.001
	B.L -DAY 45	0.73667	0.51465	0.017	0.87000	0.42249	0.002
	B.L -DAY 90	0.65333	0.44212	0.015	0.72143	0.38255	0.002
B.l	B.L -DAY 30	0.81714	0.21685	0.000	0.64571	0.20895	0.000
	B.L -DAY 45	0.73143	0.24876	0.000	0.71714	0.24985	0.000
	B.L -DAY 90	0.44571	0.26757	0.005	0.37429	0.23734	0.006

*BL- Baseline *L.f – *Lactobacillus fermentum* *B.l – *Bifidobacterium longum*

Table 2. Intragroup comparison of Gingival index from baseline to 90 days.

Gingival İndex		Diabetic Group			Non-Diabetic Group		
		Paired diff		.sig	Paired diff		.sig
		Mean	S.D		Mean	SD	
Placebo	B.L -DAY 30	0.83571	0.23187	0.000	0.60333	0.40033	0.014
	B.L -DAY 45	0.83571	0.23187	0.000	0.60333	0.40033	0.014
	B.L -DAY 90	0.82000	0.23144	0.000	0.45333	0.54080	0.095
L.f	B.L -DAY 30	0.45667	0.39808	0.038	0.85000	0.43768	0.002
	B.L -DAY 45	0.45667	0.39808	0.038	0.85857	0.42928	0.002
	B.L -DAY 90	0.45667	0.39808	0.038	0.70000	0.54708	0.015
B.l	B.L -DAY 30	0.47571	0.38043	0.016	0.65429	0.43749	0.007
	B.L -DAY 45	0.31286	0.59930	0.216	0.61857	0.41875	0.008
	B.L -DAY 90	0.47571	0.38043	0.016	0.27000	0.31596	0.064

*BL- Baseline *L.f – Lactobacillus fermentum *B.l – Bifidobacterium longum

Table 3. Intragroup comparison of Gingival Bleeding index from baseline to 90 days.

Gingival Bleeding İndex		Diabetic Group			Non-Diabetic Group		
		Paired diff		.sig	Paired diff		.sig
		Mean	S.D		Mean	SD	
Placebo	B.L -DAY 30	13.85714	5.04739	0.000	17.66667	9.75021	0.007
	B.L -DAY 45	14.42857	5.28700	0.000	18.66667	11.14750	0.009
	B.L -DAY 90	14.71429	5.43796	0.000	18.33333	11.27239	0.010
L.f	B.L -DAY 30	16.50000	6.18870	0.001	17.14286	7.19788	0.001
	B.L -DAY 45	16.83333	6.70572	0.002	18.00000	8.48528	0.001
	B.L -DAY 90	17.50000	6.97854	0.002	17.71429	8.82637	0.002
B.l	B.L -DAY 30	18.57143	13.83061	0.012	20.57143	6.50275	0.000
	B.L -DAY 45	18.85714	13.81338	0.011	20.57143	6.50275	0.000
	B.L -DAY 90	17.28571	14.93000	0.022	19.71429	7.52140	0.000

*BL- Baseline *L.f – *Lactobacillus fermentum* *B.l – *Bifidobacterium longum*

Table 4. Intragroup comparison of Probing pocket depth from baseline to 90 days.

Probing Pocket Depth		Diabetic Group			Non-Diabetic Group		
		Paired diff		.sig	Paired diff		.sig
		Mean	S.D		Mean	SD	
Placebo	B.L -DAY 30	1.14286	0.69007	0.005	1.33333	0.81650	0.010
	B.L -DAY 45	1.28571	0.48795	0.000	1.66667	0.51640	0.001
	B.L -DAY 90	1.14286	0.69007	0.005	1.33333	0.81650	0.010
L.f	B.L -DAY 30	1.50000	0.54772	0.001	1.42857	0.78680	0.003
	B.L -DAY 45	1.66667	0.51640	0.001	1.28571	0.75593	0.004
	B.L -DAY 90	1.66667	0.51640	0.001	1.28571	0.75593	0.004
B.l	B.L -DAY 30	1.14286	0.69007	0.005	1.14286	0.69007	0.005
	B.L -DAY 45	1.14286	0.69007	0.005	1.14286	0.69007	0.005
	B.L -DAY 90	1.14286	0.69007	0.005	1.00000	0.81650	0.018

*BL- Baseline *L.f – *Lactobacillus fermentum* *B.l – *Bifidobacterium longum*

Table 5. Intragroup comparison of total bacterial count from baseline to 30 days.

Total Bacterial Count		Diabetic Group			Non-Diabetic Group		
		Paired diff		.sig	Paired diff		.sig
		Mean	S.D		Mean	SD	
Placebo	B.L -DAY 30	31.57482	52.66532	0.17	8.56667	11.00430	0.115
L.f	B.L -DAY 30	182.37350	347.73634	0.045	145.96357	166.88299	0.040
B.l	B.L -DAY 30	335.10929	357.65829	0.048	149.77657	174.71266	0.046

*BL- Baseline *L.f – Lactobacillus fermentum *B.l – Bifidobacterium longum

Table 6. Inter and intragroup comparison of P.g and A.a from baseline to 30 days

	Diabetic		Non-Diabetic	
	Mean ± SEM	P- value	Mean ± SEM	P- value
P.g	-41.67 ± 2.494	0.679	-35.00 ± 1.291	0.40
A.a	-37.33 ± 1.453	0.737	-20.00 ± 1.826	0.60
Sub-Groups (*P.g + A.a*)				
placebo	-11.67 ± 2.848	0.329	-8.000 ± 1.291	0.4
L.f	-50.67 ± 2.603	0.426	-22.67 ± 2.963	0.785
B.l	-33.00 ± 2.887	0.720	-12.00 ± 2.582	0.4

*P.g – Porphyromonas gingivalis * A.a – Aggregatibacter actinomycetemcomitans
*BL- Baseline *L.f – Lactobacillus fermentum *B.l – Bifidobacterium longum

Discussion

Studies have revealed a possible link between systemic diseases and periodontitis. It was accepted that people with diabetes are more prone to establish periodontal diseases. Similarly, periodontal disease might be a risk factor for diabetes.[13] There is also evidence indicating that oral bacteria play an essential role in diabetes and obesity. Direct association between *A.actinomycetamcomitans, P.gingivalis,* and glycemic control was reported in a few studies.[14] These pathogens were also believed to cause dysbiosis in gut microbiota,[15] altering glucose metabolism. So, the treatment of periodontal disease in diabetic subjects should also focus on lowering blood glucose levels, which might act as an adjuvant to conventional periodontal therapy.

Bacterial therapy, in the form of probiotics, offers a dual role in maintaining gut health as well as reducing the colonization of oral bacteria. Anti-diabetic effects of probiotics are due to their competitive inhibition, immunomodulation, antioxidant, and anti-inflammatory properties.[16] An analogous mechanism occurs in the oral cavity and intestine when probiotics are consumed. In the oral cavity, probiotics directly engage in the metabolism of bacterial substrates and inhibit bacterial colonization. They compete and intervene with bacterial attachments and prevent plaque formation.[17]

Lactobacillus fermentum is a ubiquitous, gram-positive, fermentative bacteria and helps in the production of enzymes that metabolize carbohydrates proteins and break down bile salts.[18] *Bifidobacterium longum* is an anaerobe, predominates in the large intestine[19], and is also present in the oral cavity of healthy subjects. They metabolize lactose and ferment indigestible carbohydrates.[19]

To our knowledge, this is the first study comparing the efficacy of *Lactobacillus* and *Bifidobacterium* species among diabetic and non-diabetic subjects with periodontitis. After screening, 48 subjects were selected for the study, and informed consent was taken. Due to the COVID-19 pandemic, 8 subjects were dropped out of the study, and 40 subjects (20-diabetic, 20-non-diabetic) were analyzed for final results. Chewing gum was selected for carrying probiotics with the aim that 'functional foods' which have better compliance should become a part of the treatment of periodontal diseases.

When considering changes in mean PI, a significant reduction was observed in both groups and also within the subgroups. From day 30 to day 90, there is a trend of a mild increase in PI in all the groups and subgroups, which was not statistically significant. For PI, the mean difference from baseline to 90 days in placebo, L.f, B.l are 0.36±0.27, 0.7±0.4, 0.41±0.25, respectively, which shows statistically significant difference within the subgroups(p=.028). The above mean difference stated that *Lactobacillus fermentum* is more effective in controlling plaque at the end of 90 days, followed by *Bifidobacterium longum*.

On intergroup and intragroup comparison of mean GI, Gingival bleeding Index, and PPD, a significant reduction was observed from baseline to 30 and 45 days. At the end of day 90, the *Bifidobacterium longum* subgroup showed an increase in mean GI (Diabetic and Non-diabetic groups) and gingival bleeding (Diabetic group), which is statistically significant.

A study by Sabatini *et al*. assessed the efficacy of *L. reuteri* tablets on gingivitis subjects with diabetes.[20] SRP was not performed, and subjects were asked to use probiotic pills twice a day for 30 days. A significant change

was observed in only GI. So, this study proved that probiotics act better when used as an adjuvant to SRP. Szkaradkiewicz *et al*. provided *L. reuteri* lozenge to subjects with Periodontitis two times/day for 14 days after SRP.[21] A significant reduction was observed only in BOP, PPD, CAL, and GCF biomarkers in the probiotic group. PI and GI were not reduced significantly. In our present study, when probiotics are given two times/day after SRP for 30 days, all clinical parameters were significantly reduced. There is no evidence showing the exact dosage of probiotics required for maintaining oral health. Most of the studies used 10^8 CFU/ml of probiotics for different time periods. Each chewing gum contained 10^8 CFU in this study and was taken twice daily for 30 days.

Microbial analysis showed no significant difference in the total bacterial count for intergroup comparison but a considerable reduction within the subgroups(p=0.048). Probiotic subgroups of diabetic and non-diabetic groups revealed a statistically significant decrease in the bacterial count, and control groups non-significant reduction.

It was believed that supragingival plaque control affects the subgingival microbial environment by reducing pocket depth in advanced supragingival lesions but not in the case of angular bone defects and deep pockets.[22] However, the mean PPD of the present study at baseline is 6.7±0.98 in the diabetic group and 6.6±1.05 in the non—diabetic group, which indicates that subjects with moderate periodontitis were included in the study.

In the PCR analysis of subgingival plaque samples, it was observed that the prevalence of P.g and A.a before treatment is slightly more remarkable in the diabetic group, but the difference is not significant(graphs 3A and 3B). Intragroup analysis of PCR revealed that L.f and B.l in the diabetic group showed a three-fold decrease of *P.g* and *A.a* while placebo showed only a one-fold decrease. In the non-diabetic group, placebo and *L.f* showed a two-fold decrease of *P.g* and *A.a* while *B.l* showed only a one-fold decrease. However, the reduction is not significant in any of the subgroups for both *P.g* and *A.a* when evaluated 30 days after using probiotics.

Randomized control trial by Invernici *et al.* evaluated the effect of *Bifidobacterium lactis* lozenges on chronic periodontitis when taken twice/day for 30 days as an adjunct to SRP. At the end of 30 days, no difference was observed between test and control groups for red-complex bacteria, whereas this percentage has reduced significantly after 90 days in the test group. This could be explained by probiotics that might have acted delaying in the recolonization of pathogens in periodontal pockets.[23] In the present study, after 30 days, there is no significant reduction in *P.g* and *A.a,* which might be explained by the same.

Chen *et al*. reported that *Lactobacillus fermentum* showed more potent inhibitory effects on *Porphyromonas gingivalis.*[24] The mechanism can be explained that probiotics produce organic acids, which decrease the pH oxidation-reduction potential and inhibits the growth of pathogenic bacteria. Hojo *et al*. explained that *B.longum* competes with *P.gingivalis* for salivary vitamin K, which is their mutual growth factor.[25]

In the present study, there is no significant difference in clinical or microbial parameters between diabetic and non-diabetic groups. The intragroup comparison significantly reduced all clinical parameters from baseline to 30,45 and 90 days. A substantial decrease in the total bacterial count was observed in probiotic groups but not in placebo. PCR analysis showed a non-significant reduction of *P.g* and *A.a* in all the subgroups.

This study has certain limitations, such as a small sample size and less follow-up, and HbA1C levels of diabetic subjects were not evaluated post-treatment.

Conclusions

Probiotic functional foods can be delivered as an adjunct to SRP for the management of periodontitis in systemically compromised subjects. Long term use or inclusion of probiotics in the diet is recommended to maintain recolonization of bacteria.

Acknowledgements

Heartfelt thanks to CFTRI, Mysuru for providing probiotic culture, JSS Dental College, and Hospital for providing all the facilities required to carry out research, and all the participants of the study.

Conflicts of Interest Statement

No conflicts of interest.

References

1. Janakiram C, Mehta A, Venkitachalam R. Prevalence of periodontal disease among adults in India: A systematic review and meta-analysis. J Oral Biol Craniofacial Res [Internet]. 2020;10(4):800–806.
2. Botelho J, Machado V, Proença L, Mendes JJ. The 2018 periodontitis case definition improves accuracy performance of full-mouth partial diagnostic protocols. Sci Rep. 2020;10(1):1–7.
3. Chandra A, Yadav OP, Narula S, Dutta A. Epidemiology of periodontal diseases in Indian population since last decade. J Int Soc Prev Community Dent. 2016;6(2):91–96.
4. Malik G, Lehl G, Talwar M. Gaurav Malik, Gurvanit Lehl, Manjit Talwar. Association of Priodontitis with diabetes mellitus: a review.J of medical college chandigarh;2011;1(1).
5. Care D, Suppl SS. 2. Classification and diagnosis of diabetes: Standards of medical care in diabetes-2021. Diabetes Care. 2021;44(January):S15–33.
6. Preshaw PM, Alba AL, Herrera D, Jepsen S, Konstantinidis A, Makrilakis K, et al. Periodontitis and diabetes: A two-way relationship. Diabetologia. 2012;55(1):21–31.
7. Jindal V, Mahajan N, Goel A, Kaur R, Mahajan A, Malhotra P. Clinical efficacy of probiotic mouthwash in the treatment of gingivitis patients in Himachal population. J Int Clin Dent Res Organ. 2017;9(1):41.
8. Hill C, Guarner F, Reid G, Gibson GR, Merenstein DJ, Pot B, et al. Expert consensus document: The international scientific association for probiotics and prebiotics consensus

statement on the scope and appropriate use of the term probiotic. Nat Rev Gastroenterol Hepatol. 2014;11(8):506–514.

9. Nguyen T, Brody H, Radaic A, Kapila Y. Probiotics for periodontal health—Current molecular findings. Periodontol 2000. 2021;87(1):254–267.

10. Kocsis T, Molnár B, Németh D, Hegyi P, Szakács Z, Bálint A, et al. Probiotics have beneficial metabolic effects in patients with type 2 diabetes mellitus: a meta-analysis of randomized clinical trials. Sci Rep [Internet]. 2020;10(1):1–14.

11. MP S, Bhatia A. Role of functional foods in periodontal health and disease. Indian J Dent Adv. 2011;03(03):587–592.

12. Boutaga K, Van Winkelhoff AJ, Vandenbroucke-Grauls CMJE, Savelkoul PHM. Periodontal pathogens: A quantitative comparison of anaerobic culture and real-time PCR. FEMS Immunol Med Microbiol. 2005;45(2):191–199.

13. Arigbede AO, Babatope BO, Bamidele MK. Periodontitis and systemic diseases: A literature review. J Indian Soc Periodontol. 2012;16(4):487-491.

14. Long J, Cai Q, Steinwandel M, Hargreaves MK, Bordenstein SR, Blot WJ, et al. Association of oral microbiome with type 2 diabetes risk. J Periodontal Res. 2017;52(3):636–643.

15. Olsen I, Yamazaki K. Can oral bacteria affect the microbiome of the gut? J Oral Microbiol [Internet]. 2019;11(1).

16. Gomes AC, Bueno AA, De Souza RGMH, Mota JF. Gut microbiota, probiotics and diabetes. Nutr J. 2014;13(1).

17. Anusha RL, Umar D, Basheer B, Baroudi K. The magic of magic bugs in oral cavity: Probiotics. J Adv Pharm Technol Res. 2015;6(2):43–47.

18. Patil MB, Reddy N. Bacteriotherapy and probiotics in dentistry (2006). KSDJ; 2:98-102.

19. Galdeano CM, de Moreno de LeBlanc A, Vinderola G, Bonet ME, Perdigón G (2007). Praposed model: mechanisms of immunomodulation induced by probiotic bacteria. Clinical and Vaccine Immunology; 14:485-492.

20. Sabatini S, Lauritano D, Candotto V, Silvestre FJ, Nardi GM. Oral probiotics in the management of gingivitis in diabetic patients: A double blinded randomized controlled study. J Biol Regul Homeost Agents. 2017;31(2, Supplement 1):197–202.

21. Szkaradkiewicz AK, Stopa J, Karpiński TM. Effect of oral administration involving a probiotic strain of Lactobacillus reuteri on pro-inflammatory cytokine response in patients with chronic periodontitis. Arch Immunol Ther Exp (Warsz). 2014 Dec;62(6):495-5.

22. Hellström MK. The effect of supragingival plaque control on the subgingival microflora in human periodontitis. J Clin Periodontol. 1996;23(10):934–940.

23. Invernici MM, Salvador SL, Silva PHF, Soares MSM, Casarin R, Palioto DB, et al. Effects of Bifidobacterium probiotic on the treatment of chronic periodontitis: A randomized clinical trial. J Clin Periodontol. 2018;45(10):1198–1210.

24. Chen LJ, Tsai HT, Chen WJ, Hsieh CY, Wang PC, Chen CS, et al. In vitro antagonistic growth effects of lactobacillus fermentum and lactobacillus salivarius and their fermentative broth on periodontal pathogens. Brazilian J Microbiol. 2012;43(4):1376–84.

25. Hojo K, Nagaoka S, Murata S, Taketomo N, Ohshima T, Maeda N. Reduction of vitamin K concentration by salivary Bifidobacterium strains and their possible nutritional competition with Porphyromonas gingivalis. J Appl Microbiol. 2007;103(5):1969–1974.