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Orally administered probiotics (Lactobacillus Brevis CD2) lozenges in chronic periodontitis patients among smokers and non-smokers – A clinical and microbiological study

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Abstract

Objectives: A parallel designed uncentered study was planned to compare the clinical and microbiological outcomes of scaling and root planing with adjunctive probiotic administration between smokers and non-smokers with chronic periodontitis.

Method: 30 patients with chronic periodontitis (15 smokers and 15 non-smokers) who satisfied the inclusion and exclusion criteria were enrolled in the study. Groups underwent full mouth scaling and root planing followed by administration of Lactobacillus Brevis CD2 lozenges. Plaque index, gingival index, probing pocket depth and relative attachment level were the clinical parameters assessed. Subgingival plaque samples were evaluated for microbiologic analysis using total anaerobic count for non-specific microbial evaluation and RT-PCR for specific microbial analysis of Porphyromonas gingivalis and Tanerella forsythia. The clinical parameters and colony-forming units were evaluated on the 30th day, 60th day, and 90th day. The RT-PCR analysis was carried out at baseline, 60th day, and 90th day. Statistical analysis of the data was performed. (p-value < 0.05)

Results: In the microbiologic analysis both the groups showed a statistically significant reduction in the specific microbial count from baseline to end of treatment intervention except for smokers for whom reduction in Tannerella forsythia was not maintained till the end of treatment. However, on intergroup analysis a statistically significant difference was seen between smokers and non-smokers with respect to plaque index, probing pocket depth, relative attachment level, and microbiologic analysis with a p-value < 0.05

Conclusion: The present study showed that probiotics when used as adjuvants to be scaling and root planing improved the periodontal status even in presence of smoking.

Keywords: chronic periodontitis, probiotics, scaling and root planing, smoking, microbial analysis, RT-PCR, lactobacillus brevis CD2.

Introduction

Periodontitis is a chronic inflammatory disease of the tooth-supporting tissues. **[1]** Recent metagenomic, meta transcriptomic, and mechanistic studies have put forth a new model of periodontal disease pathogenesis has been upraised which suggests that periodontal disease may arise due to polymicrobial synergy and dysbiosis, which perturb the ecologically balanced biofilm associated with periodontal tissue homeostasis. **[2]** With this background now the treatment strategy for periodontal disease has been shifted towards modifying the pathological plaque to a biofilm of commensalisms.**[3]** Probiotics or the health-beneficial bacteria to treat oral diseases after many years of their successful utilization in gastrointestinal disorders. The principal treatment of periodontal therapy is mechanical plaque debridement which is believed to temporarily shift the subgingival flora to a less pathogenic composition in about 3 weeks returning to baseline values. Hence, lately, the focus of treatment strategy has been shifting towards the use of probiotics which not only suppress the emergence of endogenous pathogens or prevent superinfection with exogenous pathogens, they are capable of promoting beneficial host response.**[4]** Probiotics can bring about improvement in periodontal clinical conditions, reduce the load of pathogenic microorganisms and alter the host immune response as well.**[5,6]** Lactobacillus brevis CD2 is a probiotic form that has gained its

application into periodontal usage recently presenting improved periodontal conditions in both clinical and microbiological arenas.**[7]** According to the World Health Organisation, the number of smokers worldwide are more than 1 billion and is expected to increase to 1.7 billion by 2025.**[8]** Smoking, a most important preventable risk factor for periodontitis, is demonstrated by several epidemiological studies. **[9]** Scaling and root planning are not successful in maintaining long-term success among smokers as the clinical outcome after periodontal therapy depends upon a suitable reduction in periodontal pathogens. Therefore, there is a need for the development of more efficient periodontal therapies for smokers.**[10]**

The present study is an attempt to evaluate the efficacy of Lactobacillus Brevis CD2 containing probiotic lozenges (1X108 million CFU) among smokers and non-smokers and its effect on the clinical and microbiological parameters when used as an adjunct to be scaling and root planning in patients with chronic periodontitis.

Materials and methods

Source of data

A total of 30 patients including 15 smokers and 15 non-smokers with an age range of 25-60 years were considered for the study. Ethical clearance was obtained from the ethical committee of Krishnadevaraya College of Dental Sciences and Hospital, affiliated with the Rajiv Gandhi University of Health Sciences. Informed consent was obtained from each patient who was willing to take part in the clinical trial. (NCT02329353)

Inclusion criteria: Patients free from any systemic illness, previously untreated moderate to severe generalized chronic periodontitis, patients who have not participated in any of the clinical trials during the previous 4 weeks, patients not using any of the probiotic supplements, patients free from adverse reactions to lactose or fermented milk products, patient unwilling to quit smoking and smokers over the past one year. Exclusion criteria: Previous history of antibiotic usage over the past 6 months, acute oral lesions or necrotizing ulcerative periodontitis, and patients with any other systemic disease.

Subjects grouping:

Group I – 15 smokers with chronic periodontitis having a probing pocket depth of ≥ 5 mm with bleeding on probing positive and radiographic evidence of bone loss in at least two sites in each quadrant.

Group II – 15 Non-smokers with chronic periodontitis having probing pocket depth of ≥ 5 mm with bleeding on probing positive and radiographic evidence of bone loss in at least two sites in each quadrant.

Study Design

The site with the deepest probing pocket depth (PPD) was selected as a test site. Each subject underwent full-mouth periodontal probing, measured on six sites (distobuccal, mid-buccal, mesiobuccal, disto-lingual, mid-lingual, and mesio-lingual) per tooth using a UNC-15 periodontal probe. Clinical parameters were recorded at baseline, 4th week, 8th week and 12th week follow up: Plaque index (Sillness and Loe, 1964), Gingival index (Loe and Sillness, 1963), Probing pocket depth, and Relative attachment level using customized acrylic stents were recorded. Dental plaque samples were collected from this test site at baseline, 30th day, 60th day, and 90th day follow-up visits. Supragingival plaque and calculus were removed using sterile standard periodontal scalers to permit the easy collection of the subgingival plaque samples. Two subgingival plaque samples were collected from the selected tooth using a sterile curette with an upward stroke. The collected samples were placed in a TE buffer solution (Tris- HCl 10mm EDTA 1mm, pH 7.6) and were sent for microbiological analysis. The first plaque sample was sent for Real-time Polymerase Chain Reaction (RT-PCR) analysis for Porphyromonas gingivalis (PG) and Tannerella forsythia (TF) and the second set of samples were sent for total anaerobic count using bacterial culture technique. This was followed by scaling, root planning, and oral hygiene instructions. The probiotic lozenges containing Lactobacillus brevis CD2 in the concentration of 1x108 CFU were distributed among both the test groups at the baseline and were instructed to take a dosage of three lozenges per day, one in the morning and two at night for 60 days. This was considered as baseline. All the subjects were recalled again on the 30th day, 60th day, and 90th day for subgingival plaque samples collection analyzed for colony forming units (CFU) using bacterial culture technique. Plaque samples collected on the 60th day and 90th days were analyzed for PG and TF using Real-time PCR. Thus, a total of 120 samples were collected for analyzing total anaerobic colony forming units (CFU) and 90 samples were collected for RT-PCR analysis.

Microbiologic analysis

The samples that were collected from the patients were transferred to TE buffer solution and incubated for two hours. The samples were then serially diluted, 100 microliters of the diluted specimen were streaked onto blood agar supplemented with hemin (5mg/ml) and vitamin k (10mg/ml) and anaerobically cultured using an anaerobic jar at 35-370 C for 2-3 days. All samples were inspected for total anaerobic CFUs using the digital colony counter.

PCR Design and synthesis

The primers for quantification analysis were designed using Perkin Elmer Primer Express® software. The Melting temperature (Tm) was calculated, and the synthesized primers were purified by HPLC. The quantified DNA was used to detect the presence of Porphyromonas gingivalis (PG) and Tanarella forsythia (TF) using the specific primers below and Primer optimization was done in a gradient PCR

and found the annealing temperature as 60oC. Quantification was performed in Applied Biosystems StepOne Real-Time PCR (Foster City, CA). All reaction components are procured from Life Technologies. Standard reaction volume 10 µl contains 1X Taq man-PCR buffer, 3 mM MgCl2, 0.2 mM each of dATP, dCTP, dGTP, 0.4 mM dUTP, 0.005 U AmpliTaq Gold, 0.002 U. AmpErase UNG erase enzyme, 0.35 µl DNA template and 50–900 nM of oligonucleotide primer. The initial steps of RT-PCR were 2 min at 50°C for UNG erase activation, followed by a 10 min hold at 95°C. Cycles (n = 40) consisted of a 15-sec melt at 95°C, followed by a 30-sec annealing/extension at 55°C. The final step was 60°C incubation for 30 sec for an extension. All reactions were performed in duplicates against a serially diluted standard. Amplicons of POG cloned into the plasmid were used as a standard for the quantification of the sample. Threshold cycle (Ct) analysis of all samples was either set at 0.5 relative fluorescence units or left to automatic detection by the system.

Absolute quantification analysis

A standard curve with the highest R2 value was constructed based on the values generated by the qPCR and the quantity of POG in each sample was calculated against the standard values.

Statistical Analysis:

The results for each parameter (numbers and percentages) for discrete data and mean and standard deviation for continuous data were calculated. The normality assumption of data was tested using the Shapiro Wilks test. For data with normal distribution student-t-test was performed and for those not following normal distribution Man-Whitney U test was performed. Group sample sizes of 12 to achieve 90 % power to detect a difference of -1.1 between the null hypothesis that both groups mean are 2.5 and the alternative hypothesis that the mean of group 2 is 3.6 with estimated group standard deviations of 0.5 and 1.1 and with a significance level (alpha) of 0.05 using a two-sided Mann- Whitney test assuming that the actual distribution is uniform. However, group samples with 15 each were considered in the present study.

Results

The study population comprised 15 smokers with a mean age of 35.87 years and 15 non-smokers with a mean age of 38.47 years, which was not statistically significant. Clinical parameters assessed included PI, GI, PPD, and RAL.

PI scores between different time intervals i.e., baseline, 30th day, 60th day, and 90th day showed highly statistical significance for both smokers and non-smokers. Non-smokers showed a higher reduction than smokers. The difference between the groups was statistically significant. GI scores also showed similar results when assessed between intervals with $p < 0.001$. Differences in GI score reduction following treatment intervention between smokers and non-smokers were not significant. It was statistically significant only when compared between baseline to 90th day for smokers and highly significant from baseline to subsequent visits for non-smokers. Difference in reduction was also statistically significant with a higher amount of reduction seen among non-smokers. **(Table 1)** Probing pocket depth reduction was considered as the primary variable and was significant among non-smokers. Statistically, significant differences could be noted between both the groups in the present study. **(Figure 1)** The reduction in RAL from baseline to subsequent visit was statistically significant only among non-smokers at 60th and 90th day recall visits. The difference between the groups was statistically significant ($p < 0.01$).

Table 1: Inter-group comparison of plaque and gingival index at baseline, 30th day, 60th day and 90th day, by using student t test.

Plaque Index								
Visit		**N**	**Mean**	**SD**	**Min.**	**Max.**	**'t' value**	**'p' value**
Baseline	**Smokers**	15	1.97	0.202	1.60	2.16	7.518	0.011*
	Non-Smokers	15	1.76	0.220	1.25	2.00		
30thDay	**Smokers**	15	1.39	0.156	1.00	1.58	25.021	0.014*
	Non-Smokers	15	1.14	0.122	1.00	1.33		
60thDay	**Smokers**	15	1.47	0.160	1.08	1.66	23.087	0.028*
	Non-Smokers	15	1.22	0.124	1.00	1.40		
90thDay	**Smokers**	15	1.58	0.167	1.25	1.83	36.183	0.045*
	Non-Smokers	15	1.25	0.131	1.00	1.42		
Gingival Index								
Baseline	**Smokers**	15	1.43	0.119	1.20	1.60	26.810	0.005*
	Non-Smokers	15	1.71	0.171	1.45	2.00		
30thDay	**Smokers**	15	1.10	0.067	1.00	1.20	1.007	0.324
	Non-Smokers	15	1.14	0.122	1.00	1.45		

60thDay	**Smokers**	15	1.14	0.085	1.00	1.25	0.647	0.413
	Non-Smokers	15	1.17	0.096	1.04	1.37		
90thDay	**Smokers**	15	1.19	0.083	1.08	1.33	0.690	0.428
	Non-Smokers	15	1.22	0.113	1.08	1.45		

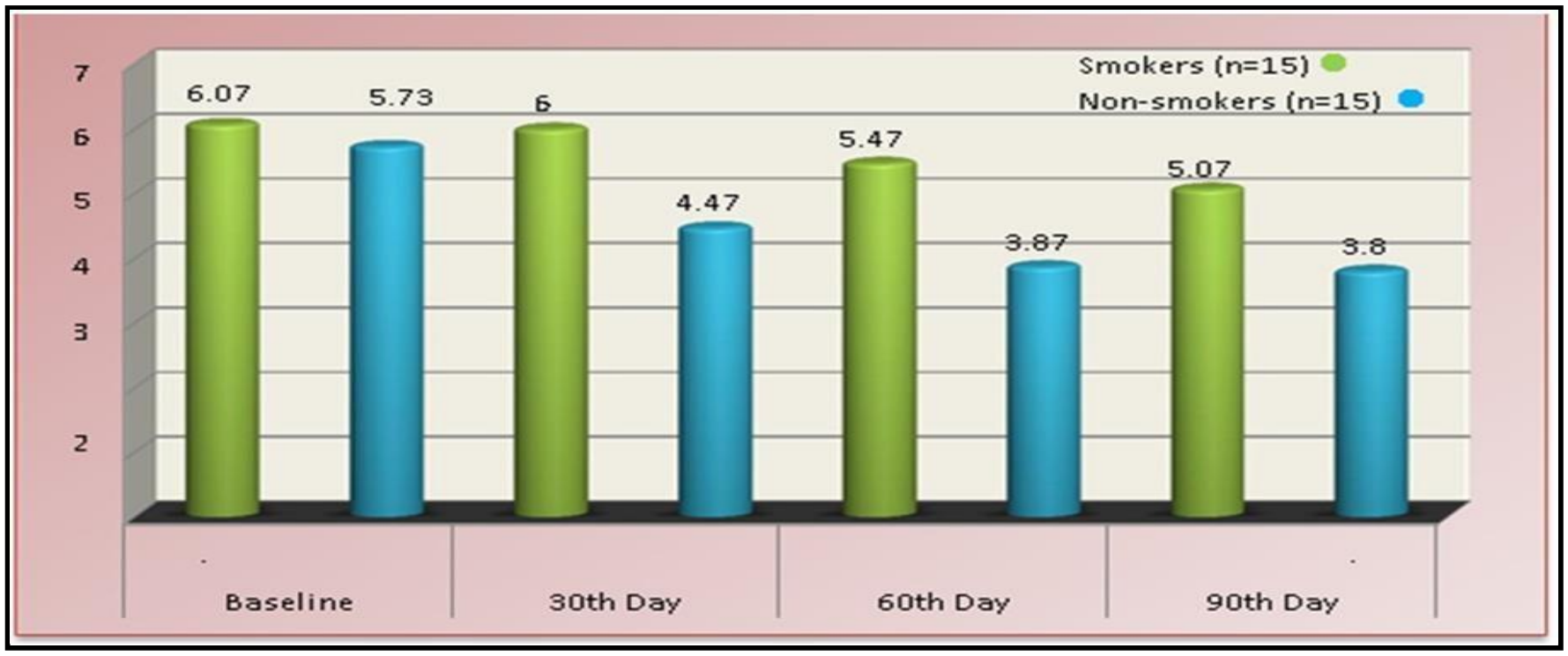

Figure 1: Inter-group comparison of Probing pocket depth at baseline, 30th day, 60th day and 90th day.

Microbiological analysis, total anaerobic colony count (CFU) between baseline CFU to subsequent visits were highly statistically significant in both groups. The difference in CFU reduction following treatment intervention was statistically significant with a higher reduction seen among non-smokers. **(Table 2)** Specific bacterial count for PG and TF was done at baseline, 60th, and 90th day using Real-Time -Polymerase Chain Reaction Analysis. A total of 90 samples were analysed in RT-PCR analysis. Specific bacterial count, PG count was highly significantly reduced at subsequent intervals in non-smokers. Smokers showed similar results. Intergroup comparison was also found to be significant. TF count was also reduced following probiotic administration in non-smokers. TF count among smokers was not statistically significant on the 90th day. The difference in reduction following treatment intervention between smokers and non-smokers was statistically significant. **(Table 3)**

Table 2: Inter-group comparison of total anaerobic colony count (in Million CFU) at baseline, 30 th day, 60th day and 90th day by using student t test.

Visit		**N**	**Mean**	**SD**	**Min.**	**Max.**	**'t' value**	**'p' value**
Baseline	**Smokers**	15	40.11	4.166	33.50	47.10	35.908	0.05*
	Non-Smokers	15	29.97	5.065	20.90	37.80		
30thDay	**Smokers**	15	24.47	2.964	20.40	29.20	52.164	0.001*
	Non-Smokers	15	16.46	3.107	11.20	22.30		
60thDay	**Smokers**	15	28.37	3.185	23.70	33.90	59.119	0.004*
	Non-Smokers	15	19.03	3.463	13.50	24.50		
90thDay	**Smokers**	15	32.53	3.610	27.10	39.20	63.273	0.006*
	Non-Smokers	15	21.68	3.857	14.50	27.50		

Table 3: Inter-group comparison of Porphyromonas Gingivalis (PG) and Tannerella forsythia (TF) levels at baseline, 30th day, 60th day and 90th day using student t test.

Porphyromonas Gingivalis								
Visit		**N**	**Mean**	**SD**	**Min.**	**Max.**	**'t' value**	**'p' value**
Baseline	**Smokers**	15	4.95	0.693	3.56	5.92	11.649	0.002*
	Non-Smokers	15	4.22	0.447	3.56	4.89		
60th Day	**Smokers**	15	3.18	0.507	1.99	3.84	24.332	0.0046*
	Non-Smokers	15	2.45	0.269	1.99	2.88		
90th Day	**Smokers**	15	4.08	0.641	2.81	5.17	35.509	0.005*
	Non-Smokers	15	2.94	0.375	2.47	3.79		
Tannerella forsythia								
Baseline	**Smokers**	15	4.08	0.750	2.14	4.96	3.196	0.011*
	Non-Smokers	15	3.68	0.451	3.17	4.56		
60th Day	**Smokers**	15	2.58	0.522	1.19	3.47	7.317	0.041*
	Non-Smokers	15	2.16	0.292	1.77	2.58		
90th Day	**Smokers**	15	3.51	0.654	1.90	4.35	16.941	0.081
	Non-Smokers	15	2.57	0.593	1.26	3.45		

Discussion

The prevailing strategies for the treatment of periodontal disease are principally guided by three factors namely the susceptible host, presence of pathogenic species, and the reduction or absence of the beneficial bacteria, which can predispose a person to develop a disease.**[8]** Probiotics can bring about balance among all these factors by their immune-modulatory, pathogenic suppression effect, and normalization of the oral ecosystem which have been previously discussed. The present study was done by administration of probiotic 'Lactobacillus Brevis CD2' among smokers and non-smokers with chronic periodontitis.

L. Brevis CD2 has been used in the treatment of a variety of ailments.**[11]** The efficacy of probiotic dosing of lozenges showed significant improvement in clinical parameters.**[12]** Probiotics lozenges were also able to reduce the plaque pH, salivary mutant streptococci, and bleeding on probing when administered for a duration of 6 weeks.**[13]** L.brevis was also evaluated in chewing gum form demonstrated a significant reduction in salivary nitrites and nitrates over subsequent visits following the intervention. Probiotics have also shown beneficial adjunctive effects (SRP) when used in combination. The proposed mechanism of action by which L. brevis has been thought to bring out its effect on the health of an individual is believed to be majorly due to arginine deaminase activity. **[12]** Association of arginine with P. gingivalis and T. forsythia, key pathogens being obligate anaerobes utilizes proteins or peptides. It is highly proteolytic enabling it to utilize free amino acids or dipeptides. L. Brevis can exert an inhibitory action on these keystone pathogens by means of their arginine deaminase activity.

By using probiotics as an adjunct to SRP, this phenomenon can be prolonged by exploiting the various known working principles of these beneficiary bacteria.**[3]** The present study was done by administering 'L. brevis CD2' among smokers and non-smokers with chronic periodontitis. Two samples of dental plaque were collected from each test site, were placed in TE buffer solution, and were sent to a laboratory for microbiological analysis. Plaque samples were collected at baseline, 30th, 60th, and 90th day follow-up visits. A total of 210 samples were collected study (120 for the total anaerobic count - CFU, 90 for RT-PCR). The result obtained was subjected to statistical analysis. L. Brevis CD2 was administered as an adjunct to SRP.

PI score in the present study showed a statistically significant reduction from baseline to subsequent visits following intervention (L. brevis CD2) is on par with previous studies. **[14-16]** Conversely, Iniesta **[17]** reported no difference in PI scores (comprised of gingivitis population). Differences observed in smokers can also be due to changes in personality traits leading to decreased oral hygiene habits, increased rate of plaque formation, or a combination of both as explained by Danielsen[18] This shows the reason for PI scores to be higher in smokers than non-smokers in our study. Intergroup examination showed a higher reduction among non-smokers which can be due to the fact that in the present study subjects who were not willing to quit smoking were enrolled as a result the subjects continued smoking throughout the study period. GI scores of the present study are similar to Vivekanada et al. (2010), Scariya et al. (2015), Tecke et al. (2015) **[14-16],** and Ince et al. (2015). **[19]** L. Brevis has also shown a reduction in the expression of inflammatory mediators such as INFγ, PGE2, metalloproteinases, and TNFa. **[20,21]** Difference in GI scores among smokers despite high plaque scores could be explained that clinical signs of inflammation are less pronounced among smokers. **[22]**

This phenomenon can result due to decreased blood vessels, gingival crevicular fluid flow, and bleeding on probing with increased inflammation.**[23]** Further on subsequent visits, no statistically significant difference between the two groups was observed. PPD acts as a reservoir of the periodontal pathogens and represents an environment with periodontal tissue destruction was considered as a primary variable in the present study. We have included relative attachment level to measure the changes in the attachment level as this could be considered a better reproducible method of measurement in absence of a clinically distinguishable CEJ.

Probing pocket reduction (PPD) from baseline to 30th day interval following SRP + probiotic showed the mean difference in PPD values was 0.067mm in smokers and 1.267mm among non-smokers. Following treatment intervention, Scariya et al. (2015) on the 30th day noted a mean reduction of 1.86mm. **[16]** Only this study had checked PPD at this time interval following treatment intervention and reported it to be 1.93mm. This is in agreement with the non-smoker group results of the present study. At the end of treatment intervention, on the 90th day, the mean difference in PPD reduction noted was 1.00mm and 1.93mm for smokers and non-smokers respectively. This reduction is in accordance with Teughels et al. (2013) **[24]**, Tekce et al. (2015) **[15]** Scariya et al. (2015) **[16],** and Ince et al., (2015) **[19]** who noted a mean difference of PPD reduction from baseline to end of treatment intervention as 1.42mm,1.93mm 1.60mm and 1.44mm respectively. PPD on intergroup examination revealed a more favorable outcome among non-smokers in relation to smokers. Reduction significance in non-smokers can be explained due to reduced response seen in general among smokers to non- surgical periodontal therapy than non-smokers which reflects itself with a reduced reduction in PPD **[25]** The gain in attachment level from baseline to 60th day was 0.333 and 1.067 among smokers and non-smokers. At the end of the treatment intervention (90th day) among smokers and non-smokers, the mean difference was 0.80mm and 1.26mm respectively. This reduction only among non-smokers is in accordance with Vivekananda et al. (2010) **[14]**, Teughels et al., (2013) **[24]**, Tekce et al., (2015) **[15]** and Ince et al. (2015) **[19]** with 1.09mm, 1.00mm, 1.18mm and 1.08mm respectively. The difference in the attachment levels between smokers and non-smokers was significant and this could be due to a severe level of attachment loss that is seen among smokers in comparison to non-smokers. Smokers are also susceptible to sustaining continued attachment loss which is six times more likely in comparison to non-smokers and the non-surgical management can result in a gain of clinical attachment that is less than non-smokers.**[26]**

Total anaerobic count (CFU) was done using the traditional culture technique. Samples were analyzed at baseline, 30^{th} day, 60^{th} day and 90^{th} day was statistically highly significant from baseline to subsequent visits. The reduction in the anaerobic bacterial load can be due to a number of defensive mechanisms exhibited by the probiotics against pathogenic organisms like alteration in the aggregation of pathogenic microorganisms **[27]**, hydrogen peroxide synthesis, synthesis of reuterin and reutericyclin,**[28]** competition for nutrients, interference with bacterial metabolism, production of short-chain fatty acids and bacteriocins.**[29]** In the present study, at the end of treatment intervention (90th day) mean difference in CFU when compared was 7.58 and 8.29 among smokers and non-smokers respectively which is similar to the results of non-smokers (26.92) in the study done by Tekce et al.2015.**[15]** Among smokers, the baseline anaerobic count was found to be higher in comparison to non-smokers. This increase in the anaerobic proportion of the bacteria can be attributed to the contribution of anaerobiosis that results from smoking. In a smoker's oral environment there is a reduction of oxidation-reduction potential which can act as a contributing factor for the progress of the destructive periodontal disease. **[30]**

Real-time polymerase chain reaction (RT PCR) is one of the sensitive methods with species-specific and sensitive primers for accurate detection of target microorganisms. **[31]** The PCR method detects both viable and non-viable bacteria. The levels of P. gingivalis (PG) were examined using RT-PCR following intervention among smokers and non-smokers. At baseline, the smokers and non-smokers presented a mean count of 4.95±0.69 and 4.22±0.44 respectively. This is the first study where the microbiological effect of the probiotic L. Brevis CD2 on total (culture technique) and specific periodontal pathogens (RT PCR) has been checked. P. gingivalis and T. forsythia were evaluated for the mean difference on the 60th day and on the 90th day, which was found to be highly statistically significant when compared to the baseline. In the present study, it was observed that there was a nearly threefold reduction in PG count and the reduction was also significant on the 90th day, it can be explained that probiotics had an effect on the microbial flora which continued even after cessation of probiotic L. brevis CD2 administration. This can be explained as arginine being an important uptake molecule of the PG **[32]** and administration of L. brevis CD2 (arginine deiminase activity) can decrease the expression of fimbrial subunits which are the key virulent determinants of PG **[33]** and thus inhibiting its role in biofilm formation. Smokers showed significantly less reduction of PG in comparison to non-smokers. This could be explained due to the less efficacy of SRP in removing the pathogenic species among smokers.**[34]** The levels of T. forsythia (TF) also showed a statistically significant reduction following treatment intervention. Intergroup comparison was done between the groups for reduction of the TF from baseline to end of the treatment intervention was different between smokers and non-smokers with greater reduction among non-smokers. It has been postulated that smokers are 2.3 times more likely to harbor TF in comparison to non-smokers. **[35]** The role of L. brevis CD2 in the reduction of TF count was found to be similar to PG count. Though in the present study an attempt was made to correlate the results with other research works, however, none of the studies involved L. brevis CD2 bacterial strain as their test component, and studies that included this probiotic strain did not

match with our study design. Further, the influence of probiotics on smokers could not be compared with Shimauchi et al. (2008) **[36]** and Mayanagi et al. (2009) **[37]** since these studies did not involve conventional periodontal intervention. These could be the reason for the differences in the results that were come across. Within the limitation of the present study was that a control group was not included which would have helped in analyzing the probiotic effect alone and the evaluation of the colonization patterns by L. brevis CD2 was not done which would have helped in determining its treatment duration. Elucidate further larger sample size and a longer duration of study to be considered.

Conclusion

Smoking cessation promoted additional benefits on non-surgical periodontal therapy in chronic periodontitis. Probiotic administration may be a biological approach for inducing a beneficial shift away from pathogens. L.brevis CD2 lozenges proved to be efficacious in reducing the rate of recolonization in smokers and significantly in non-smokers. Probiotic intervention could be used as a useful tool for treatment, especially in high-risk subjects.

Declaration of competing interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Conflict of Interest: None declared.

Authorship Contribution Statement

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Methodology: Shruthi JR. Prabhuji MLV, Rudrakshi C.

Supervision: Rudrakshi C, Prabhuji MLV, Ashwin PS.

Roles/Writing - original draft: Rudrakshi C, Ashwin PS

Writing - review & editing: Rudrakshi C, Ashwin PS& Prabhuji MLV.

Approval of a final version of the manuscript - Shruthi JR, Prabhuji MLV, Rudrakshi C & Ashwin PS.

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