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Efficacy of Probiotics As An Adjunct To Non-Surgical Periodontal Therapy (NSPT) In The Management of Chronic Periodontitis Patients - A Clinical And Biochemical Study

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Abstract

Background: Periodontal disease occurs due to an imbalance between pathogenic and beneficial oral bacteria. Although conventional periodontal therapy reduces pathogenic bacteria, it fails to restore the beneficial ones. The use of probiotics can increase the proportion of beneficial bacteria, which in turn creates a more disease-free environment. The objective of this study is to assess the effectiveness of probiotics as an adjunct to non-surgical periodontal therapy in managing patients with chronic periodontitis by evaluating salivary biomarkers.

Materials And Methods: Forty patients with chronic periodontitis participated in the study. They were randomly assigned to one of two groups: a test group receiving non-surgical periodontal therapy (NSPT) with probiotics, and a control group receiving NSPT with a placebo. Both groups underwent NSPT in two consecutive visits within a two-week period. After the NSPT, the test group received probiotic capsules twice daily for 30 days, while the control group received placebo capsules. The clinical and biochemical parameters evaluated in the study included Plaque Index, Gingival Index, Papillary Bleeding Index, Probing Pocket Depth, Clinical Attachment level, and Salivary MMP-8 and TIMP-1. The parameters were assessed at four different time points: baseline, 30th day, 60th day, and 120th day after NSPT.

Results: The study findings revealed that both the test group (NSPT with Probiotics) and the control group

(NSPT with Placebo) exhibited a significant improvement in clinical and biochemical parameters after NSPT. However, during the 60th and 120th day evaluations, the test group demonstrated a greater improvement in all parameters compared to the control group.

Conclusion: In conclusion, the study suggests that using probiotics as an adjunct to NSPT provides more significant benefits in managing chronic periodontitis patients than NSPT alone.

Keywords: Periodontal Therapy, Probiotics, Chronic Periodontitis, Salivary Biomarkers, NSPT and Placebo.

Introduction

Periodontitis is a prevalent inflammatory disease that affects the supporting tissues of teeth, leading to the destruction of alveolar bone, periodontal ligament, and cementum. The disease is caused by the accumulation of dental plaque, which consists of a complex microbial community adhering to tooth surfaces. The composition of the plaque can shift from symbiosis to dysbiosis, resulting in the development of periodontitis[1]. The host immune response plays a critical role in the pathogenesis of periodontitis, and non-surgical periodontal therapy (NSPT) is the primary treatment for chronic periodontitis. NSPT involves the mechanical removal of microbial deposits and biofilms from tooth surfaces, along with oral hygiene instruction and the use of antimicrobial agents, if necessary. Although NSPT can effectively reduce the number of periodontal pathogens and promote the resolution of inflammation, it does not fully restore the balance of the oral microbiome[2].

Probiotics are live microorganisms that confer health benefits on the host when administered in adequate amounts. The administration of probiotics can increase the proportion of beneficial bacteria and induce a more disease-free environment. Additionally, probiotics can modulate the oral microbiome by reducing the overall microbial load and restoring the balance of the microbiome. The concept of using probiotics in periodontal therapy is based on the premise that the administration of beneficial bacteria can restore the balance of the oral microbiome and reduce the inflammation associated with periodontitis[3].

Saliva is a non-invasive and easily accessible fluid that can serve as a biomarker in periodontal disease. It contains a variety of biomolecules, including enzymes, cytokines, and antibodies, that reflect the status of periodontal tissues. Salivary biomarkers have been used to evaluate the efficacy of different periodontal therapies, including probiotics as an adjunct to NSPT in the management of chronic periodontitis. MMP-8 and TIMP-1 are important salivary biomarkers for periodontal disease, reflecting the levels of inflammation and tissue destruction in the periodontium[4]. Therefore, monitoring the levels of MMP-8 and TIMP-1 in saliva can provide valuable information about the severity and progression of periodontal disease, and their changes can be used as an indicator of the effectiveness of treatment strategies.

The objective of this study is to evaluate the efficacy of probiotics as an adjunct to NSPT in the treatment of chronic periodontitis patients, and to assess their effect on salivary levels of MMP-8 and TIMP-1.

Materials And Methods

This study was designed as a single-blinded, placebo-controlled, randomized clinical trial to determine the efficiency of probiotics as an adjunct to non-surgical periodontal therapy (NSPT) in systematically healthy stage II or III chronic periodontitis patients (age group of 30 to 60 years).

All participants were provided with detailed information about the study, and informed consent was obtained

prior to enrolment. Institutional Ethical Committee and Review Board approval was obtained before the trial commenced. (Reference number: CSICDSR/IEC/0099/2019).

Sample size calculation was based on mean (SD) values obtained from relevant literature, i.e., 5.5 (0.44) and 4.65 (0.52) for the TEST and control group, respectively, with 95% power and 95% confidence interval. A minimum sample size of 20 (10 per arm) was calculated.

Inclusion criteria were based on the new consortium of case definition of chronic periodontitis (Tonetti et al, 2017), and included patients with a minimum of 20 teeth present in the oral cavity, with at least 5 teeth in each quadrant, probing pocket depth (PPD) ≥4 mm in at least two interproximal sites, clinical attachment level (CAL) ≥4 mm to 7 mm in at least two interproximal sites, and radiographically moderate horizontal bone loss evident.

Exclusion criteria were patients with antimicrobial treatment within 6 months, previous periodontal therapy within a year, systemic compromise such as diabetes, rheumatic fever, liver or kidney disease, neurological deficiencies, immunological diseases, use of medication, history of smoking, pregnancy, lactation, current use of probiotic supplements, or a history of adverse reactions to lactose or fermented milk products.

Study Design

The study design involved a randomized clinical trial with a Run-in Period, a 30-day Screening Period, a 30-day single-blind clinical trial, and a Follow-up Period for 60 days (from Day-60 to Day-120) (Figure 1)

Patients were randomly assigned to one of the two groups using the coin-flip method:

Group-I: Patients received probiotics capsules, twice daily for 30 days.

Group-II: Patients received placebo capsules, twice daily for 30 days.

Clinical parameters were recorded using a manual probe (UNC-15 probe, Hu-Friedy, Chicago, IL) and evaluated at baseline, day-30, day-60 and day-120.

The clinical periodontal parameters, evaluated were: Gingival Index (GI) - Loe.H & Silness. P in 1963, Plaque Index (PI) – Silness.P & Loe.H in 1964, Papillary Bleeding Index (PBI) – Muhlemann.H.R. and Sons in 1977, Probing Pocket Depth (PPD) and Clinical attachment level (CAL)

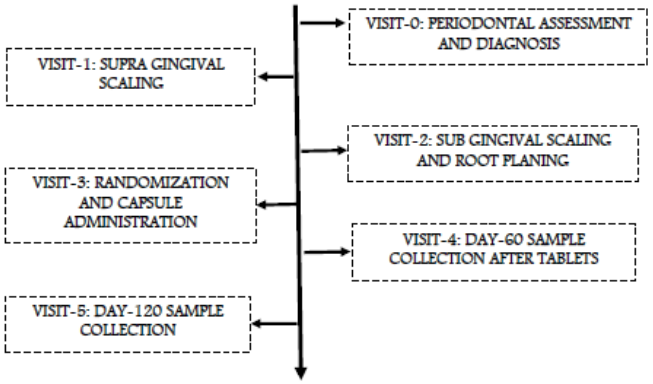

Figure 1: Study Design

Salivary Sample Collection

Biochemical parameters were evaluated by collecting whole unstimulated saliva. Prior to sample collection, all participants received written instructions detailing the procedure and necessary precautions to ensure accurate and reliable results. Participants were provided with written instructions and informed about the necessary precautions to ensure reliable results, including refraining from eating, drinking, or smoking for at least one hour prior to each sampling. Saliva was collected using the spitting method into sterile, closed containers and stored at -80°C until analysis[5]. Biochemical parameters were analysed from the collected saliva samples. (Figure 2).

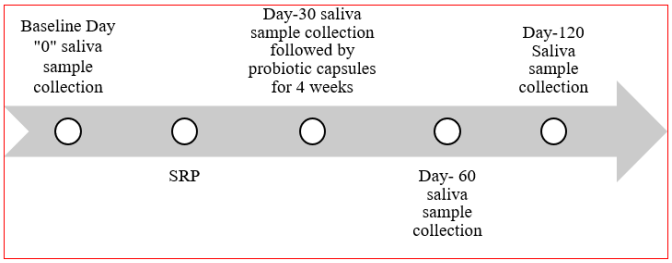

Figure 2: Sequential Salivary Sample Collection

Periodontal Therapy

A standard cycle of NSPT was performed consisting of supra- and subgingival scaling and root planing using ultrasonic scalers with fine tips and hand instruments for both the groups. NSPT was performed in two consecutive visits within a week, to ensure thorough debridement of the affected areas. To avoid any potential confounding factors that could affect the outcomes of NSPT, all the participants were instructed not to use mouthwash or gels and a modified bass brushing technique was advised. No dietary restrictions were given.

Probiotic And Placebo Administration

All the participants were instructed to take the capsules provided to them twice daily (morning and night) after food for 30 days Test group were administered probiotic capsule (Gesbact, Einoxy life sciences) that contained

Lactobacillus acidophilus-1.33 billion/spores,

Lactobacillus rhamnosus- 0.72 billion/spores,

Saccharomyces boulardii- 0.50 billion/spores

Bifidobacterium longum- 0.20 billion/spores per capsule.

Pre-measured capsules in a transparent container with patient's details given for both groups. Participants were instructed to bring the container after 30 days, to evaluate the patient compliance and adherence.

Control group were given placebo capsules that matched the following criteria:

1. Physical form- A 500mg capsule is chosen, a 0# size capsule with red cap and red capsule body. It weighed 500 ± 50mg per capsule and the water content was less than 10%. (Figure-3)

Figure 3: Probiotic and Placebo Capsule

2. Chemical nature: The placebo powder used to fill the capsule was starch. The capsule by itself was deficient of pharmacological activity with no beneficial and medicinal effects.

3. Sensory perception of the capsule and its content: Both the probiotic and the placebo capsule look alike with shiny surface and contents are matched similarly with white powder.

4. Packaging: The capsules are packed similarly in transparent plastic container. For the identification, the probiotic container had yellow cap, while placebo container had green cap.

5. Labelling: All the containers were labelled similarly. It had the patient details, number of capsules and the date of dispensing.

All clinical parameters were recorded and salivary sample was collected at day-60. Patients were advised to continue oral hygiene measures without use of mouthwash and gels. At day-120, final salivary sample was collected and clinical parameters were recorded.

Assay Procedure: Biochemical parameters Levels of MMP-8 and TIMP-1 in salivary samples were assayed by sandwich ELISA kit.

Step 1: Addition of 50 µl of Standards and 40 µl Samples to microtiter well which is pre-coated with Human TIMP-1/MMP-8 monoclonal antibody

Step 2: Addition of 10 µl of Biotin Conjugate (biotin labelled Human TIMP-1/MMP- 8 antibodies)

Step 3: Followed by addition of 50 µl of HRP Conjugate to form immune complex.

Step 4: Cover the plate and incubate for 1 hour at 37 °C in the incubator

Step 5: Unbound HRP will get removed by 1X Wash Buffer.

Step 6: Addition of Substrate A 50 µl and Substrate B 50 µl, develops blue colour during incubation period

Step 7: Reaction will stop after addition of 50 µl of Stop Solution, and will turn to yellow color.

Step 8: Read the absorbance at 450 nm using a spectrometer, within 15 minutes after adding the stop solution.

The concentration of the Human TIMP-1/MMP-8 of the sample is directly proportional to the yellow colour developed in well and will be positively correlated.

The result is plotted in a graph with standard density as the horizontal axis, OD values in the vertical axis and standard graph is drawn accordingly.

Statistical Analysis: Statistical analysis was performed using STATA version 14 software (Texas, USA), considering a P value < 0.05 as statistically significant. Continuous variables were reported as mean (SD), while categorical variables were presented as frequency (percentages). Two-sample t-tests/Mann-Whitney U tests were used to compare the test and control groups, and the Chi-Square test was utilized to assess the association between categorical variables.

Results

This study involved 40 systemically healthy patients diagnosed with chronic periodontitis who underwent nonsurgical periodontal therapy. (Table-1) They were divided into a test group (n=20) that received a probiotic capsule containing Lactobacillus acidophilus, Lactobacillus rhamnosus, Saccharomyces boulardii, and Bifidobacterium longum, and a control group (n=20) that received a placebo capsule containing starch. The capsules were taken twice daily after food for 30 days. Clinical and biochemical parameters were evaluated at baseline, day-30, day-60, and day-120.

Table 1: Demographic Data of The Patients

Parameters	Test group (n=20)	Control group (n=20)	P value
	Mean ± SD	Mean ± SD	
Age (years)	42.65±7.49	44.55±8.27	0.4514
Gender: Male	9 (45)	14 (70)	0.110
Female	11 (55)	6 (30)	

The clinical parameters evaluated were Pl-I, GI, PBI, PPD, and CAL. Intra-group comparison showed no statistically significant difference in salivary MMP-8 and TIMP-1 levels from baseline to day-120. The mean values of these parameters were statistically significant (p value <0.05) from baseline to day-120 on intergroup comparison. (Table-2)

The mean PPD and CAL values between the test and control groups at baseline were 5.52±0.52, 6.87±0.93 and 6.12±1.07, 7.34±1.09, respectively. At day-120, the mean PPD and CAL values between the test and control groups were 3.78±0.41, 5.75±0.55 and 5.83±0.96, 7.04±1.13, respectively. The inter-group comparison of these variables showed that PPD and CAL were statistically significant.

The mean MMP-8 and TIMP-1 values between the test and control group at baseline were 1.94±0.49, 1.93±0.63 and 2.34±0.69, 1.91±0.73, respectively. At day-120, the

mean MMP-8 and TIMP-1 values between the probiotics and placebo groups were 0.7±0.31, 1.64±0.32 and 2.32±0.53, 1.59±0.44, respectively.

Table 5: Inter-Group Comparisons of Clinical And Biochemical Parameters At Day-30, Day-60 And Day-120

Parameters	BASELINE		DAY-30		DAY-60		DAY-120		P-Value*
	Test Group	Control Group	Test Group	Control Group	Test Group	Control Group	Test Group	Control Group	
	Mean ± SD	Mean ± SD	Mean ±S D	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	
PI	2.36±0.5	2.67±0.34	0.38±0.2	0.47±0.2	0.29±0.17	0.88±0.37	0.64±0.25	2.15±0.44	<0.0001
GI	2.46±0.49	2.74±0.31	0.31±0.15	0.42±0.21	0.22±0.16	0.92±0.48	0.54±0.28	2.15±0.44	<0.0001
PBI	3.08±0.81	3.39±0.5	0.3±0.19	0.53±0.41	0.22±0.16	1.27±0.71	0.45±0.25	2.19±0.36	<0.0001
PPD	5.52±0.52	6.12±1.07	3.9±0.28	5.54±0.94	3.64±0.34	5.56±0.9	3.78±0.41	7.04±1.13	<0.0001
CAL	6.87±0.93	6.75±1.11	5.7±0.6	6.7±1.1	5.58±0.57	6.64±1.04	5.75±0.55	7.04±1.13	<0.0001
MMP-8	1.94±0.49	2±0.64	1.53±0.49	2±0.64	0.94±0.39	2.07±0.53	0.7±0.31	2.32±0.53	<0.0001
TIMP-1	1.93±0.63	1.84±0.46	1.69±0.28	1.84±0.46	1.55±0.34	1.89±0.5	1.64±0.32	1.59±0.44	<0.0001

Discussion

The aim of this study is to assess the effectiveness of probiotics as an adjunct to non-surgical periodontal therapy, specifically scaling and root planing, in managing patients with chronic periodontitis. As periodontal microbiota is typically commensal, existing in a state of symbiosis with the host and environment, disease expression and progression result from a complex interplay between the bacteria, the host, and the environment (Cullinan et al., 2001). Thus, an understanding of the etiology and pathogenesis of periodontal disease is crucial for treatment planing. The critical factor in periodontal therapy is not the treatment modality, but recognizing the multifactorial nature of periodontal disease and the individual patient variability.

Contemporary periodontal therapy mainly focuses on establishing a clinically and biologically stable periodontium that is both practical and individually achievable. Connie L Drisko (2014) reported that periodontal debridement is a safe and gold-standard modality for removing subgingival plaque and calculus in the treatment of periodontal disease.[6]

Although NSPT is considered standard therapy, the effect of the therapy is short-term with eventual recolonization of periodontopathogens at the treated sites. This led to the development of newer therapies that advocated both control of pathogenic bacteria and improvement of the host immune state. The use of systemic antimicrobials, local drug delivery, host modulation, photodynamic therapy, and probiotic administration are some of the therapies that are considered as adjuncts to scaling and root planing. The adjunctive use of probiotics has gained spotlight in recent times, which confers a combined effect of reducing pathogenic bacteria and host modulation.

In general, systemic administration of probiotics helps to improve overall general health as well as oral health either by modulating host mechanisms or altering the bacterial composition. Various studies are available in literature to support the positive effects of probiotics on gingival and periodontal health.

Teughels W, Loozen G, Quirynen M (2011) stated that probiotics can influence the periodontal microbiota and improve periodontal health. It produces a synergistic effect along with conventional periodontal therapy. Combined therapy of probiotics along with nonsurgical periodontal therapy proves to be useful in the treatment of periodontal disease and maintenance of periodontal health.[7]

A meta-analysis conducted by Deborah Gruner et al (2016)[8] found that the consumption of probiotic lozenges is efficient in improving and maintaining periodontal health even in situations where oral hygiene is compromised or poor. The results of this study show that the adjunctive use of probiotics with NSPT provided better clinical improvement in the management of chronic periodontitis patients when compared to NSPT with Placebo administration. Correspondingly, the biochemical parameters (Salivary MMP-8 and TIMP-1) also exhibited significant reductions in MMP-8 level and improvement in TIMP-1 level on the 30th day, 60th day, and 120th day.

Similar results were obtained in studies conducted by Eduardo Montero et al (2017)[9] and Marcos M. Invernici et al (2018)[10], where the results demonstrated a significant difference in PI, GI, PBI, PPD, and CAL from baseline to the post-operative follow-up. The results substantiate the anti-plaque and anti-inflammatory properties of probiotics and help in maintaining periodontal health.

An RCT conducted by Vivekananda et al (2010)[11] reported a significant reduction in PI, GI, and GBI in the SRP with Probiotic group when compared to the probiotic alone and placebo groups. The effect of administered probiotic on clinical parameters was maintained until the 120th day in this study. Probiotics seem to reduce the process of re-colonization of bacteria at treated sites combined with the reduced inflammatory response of the host. The bacterial composition, host response, and environmental factors were altered and maintained at a suitable level by the probiotics. In the NSPT with Placebo group, the indices eventually reached the levels equivalent to baseline, suggesting that the recolonization of biofilm bacteria at treated sites is faster when compared with that of NSPT with Probiotic group.

Tekce M et al (2015)[12] conducted an RCT and observed significant reduction in PPD and relative gain in CAL in SRP with Probiotic lozenges group. This improvement in PPD, CAL was observed till day-180, suggesting the long-term efficacy of probiotic on periodontal health maintenance.

The immuno-modulating effect of probiotics can be evaluated by monitoring biomarkers in the oral fluid such as pro-inflammatory cytokines and anti-inflammatory cytokines. Various oral samples that can be used in the diagnosis and prediction of disease activity are GCF, oral rinse fluid, oral swab, volatiles, saliva, and plaque samples. Saliva is a better alternative for the analysis of biomarkers of health and disease. Salivary diagnostics have evolved as a sophisticated science and provide a subset for a larger field of molecular diagnostics. Various biomarkers have been identified to be associated with the diagnosis and monitoring of periodontal disease activity.

Lee et al. (2018)[13] studied the potential salivary biomarkers for monitoring the effect of non-surgical periodontal therapy. They concluded that salivary MMP-8 and IL-1β levels provide a better picture for diagnosing the level of disease activity and monitoring the effectiveness of periodontal therapy. The present study evaluates the effect of the additive use of probiotics with non-surgical periodontal therapy on the salivary levels of MMP-8 and TIMP-1. Among the two groups, the probiotic group showed a more significant decrease in MMP-8 levels and increase in TIMP-1 levels in saliva compared to the placebo group.

The result suggests that combined therapy of NSPT with probiotics provides a beneficial outcome in managing chronic periodontitis patients by reducing the inflammatory component of periodontal disease.

Supporting studies that showed similar results are Gizem Ince et al. (2015)[14], who evaluated the effects of L.reuteri along with initial periodontal therapy in the management of chronic periodontitis patients for a one-year duration, which reported a significant reduction in GCF MMP-8 and improvement in GCF TIMP-1 levels in the probiotic group, concluding the additional benefits of probiotics in improving the response rate of periodontal therapy.

Similarly, Heli Jäsberg et al. (2018)[15] reported a decrease in MMP- 8 and an increase in TIMP-1 in salivary samples after probiotic consumption. Changes in MMP-8: TIMP-1 ratio demonstrate the enhanced defensive potential achieved using probiotics.

Probiotics are suggested to be beneficial when used as a monotherapy or in combination with conventional periodontal therapy. Based on the results of this study, adjunctive use of probiotics along with non-surgical periodontal therapy proved to be beneficial in improving the clinical and biochemical outcomes of periodontal

therapy. Yet, extensive research on the long-term benefits of probiotics regarding the treatment of periodontal disease, maintenance of periodontal health, and response to periodontal therapy is needed.

Conclusion

The purpose of this study was to evaluate the efficacy of probiotics on the clinical and biochemical parameters after non-surgical periodontal therapy. Overall, the evidence suggests that probiotics may have potential benefits in the management of chronic periodontitis when used as an adjunct to NSPT. While further research is needed to establish the optimal strain, dosage, and duration of probiotic therapy, the potential for probiotics to improve clinical outcomes and restore the balance of oral microbiota makes them a promising avenue for future treatment strategies in periodontics. In conclusion, the use of probiotics as an adjunct to NSPT in the treatment of chronic periodontitis has shown promising results in improving clinical parameters and promoting bacterial balance. Within the limitation of this study, it can be inferred that administration of probiotic in combination with non-surgical periodontal therapy proves to be beneficial in treatment of periodontal disease and maintenance of optimal periodontal health.

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