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# Effectiveness of Lactobacillus Reuteri as an adjunct to Scaling and Root planing in reduction of Porphyromonas Gingivalis: A Pilot Clinical study in Stage II Grade B Periodontitis

[1]Dr Manish Gupta, [2]Dr T Prasanth, [3]Dr Shreehari AK, [4]Dr Harjeet Singh, [5]Dr Saravanan SP, [6]Dr Ruchi Harish,[7]Maj Rahul

[1-7]Department of Periodontology, Army Dental Centre (R&R), Dhaula Kuan, New Delhi.

**Corresponding Author:** Dr T Prasanth, Department of Periodontology, Army Dental Centre(R&R), Dhaula Kuan, New Delhi.

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**Abstract**

Periodontitis is a condition that arises as a alterations to the host's immune response, which are triggered by an elevated bacterial dysbiosis. This dysbiosis is primarily intensified by the presence of red complex bacteria, with Porphyromonas Gingivalis being one of them. Current treatments are centered on the nonspecific plaque hypothesis, which posits that the total elimination of plaque is vital. Probiotics, as an adjunct to non-surgical periodontal therapy, have the potential to exhibit antimicrobial effects through competitive inhibition against putative periodontal microorganisms. The purpose of this study was to evaluate the effectiveness of Lactobacillus Reuteri administered as probiotic in conjunction with scaling and root planing (SRP) in reducing the presence of Porphyromonas gingivalis (P.g) using real-time quantitative polymerase chain reaction (RT-qPCR).

**Keywords:** Probiotic, Reuteri, Microbiome, Cytoprotective

## Introduction

Periodontitis is a chronic immuno-inflammatory disease affecting tooth supporting apparatus which surrounds the teeth is primarily caused by microbiome and the host interplay in response to them.[1] The production of large spectrum of inflammatory mediators, leads to destruction of connective tissue and supporting alveolar bone. The transition from periodontal health to disease is associated with a corresponding bacterial succession from predominantly gram-positive bacterial flora to gram-negative anaerobes.

The most common anaerobic gram-negative bacteria isolated from subgingival zones are Aggregatibacter actinomycetemcomitans (Aa), Porphyromonas gingivalis (Pg), Prevotella intermedia (Pi), and Tannerella forsythea (Tf) which is commonly known as red

complex.[2]These bacteria play an important role in the onset and subsequent development of periodontitis.

At present, the most widely used form of treatment to achieve microbial reduction is the mechanical instrumentation of the root surface with hand or power-driven instruments.[3] SRP consists of mechanical removal of biofilm and is often performed along with the adjunctive use of antimicrobials which are administered locally or systemically. Studies have shown that complete eradication of periodontal pathogens is extremely difficult because of various factors like root complexities, inability to do proper instrumentation etc. Therefore, incomplete biofilm removal may lead to undesirable clinical outcomes or recurrence of periodontal disease

The combination of non-surgical periodontal therapy along with systemic or local antibiotics has been shown to enhance clinical outcome and microbiological parameters;[4] nevertheless, the global increase in antibiotic resistance is causing growing worry. Consequently, there is a lot of room for study to identify different approaches to combat microbial colonization.

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 modality 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.[6] Probiotics gets easily adapted to host as they are body's own resident flora and they are free from concerns of developing resistance and diseases.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 periodontalpathogens.[7]Probiotics inhibit the production of 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 Probiotics as an adjunct to scaling and root planing in chronic periodontitis

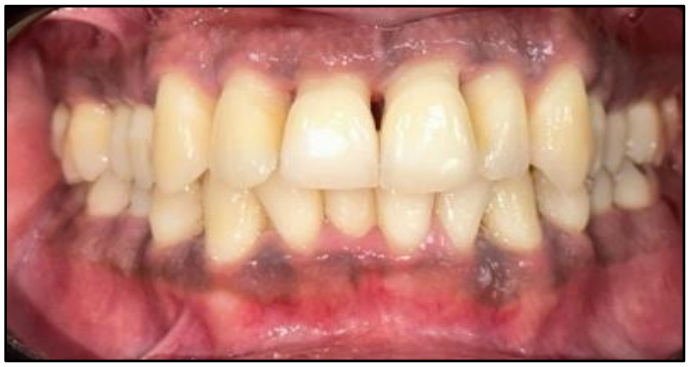
**Materials and Method**

A single blind randomized controlled, prospective clinical study was conducted for three months with 20 participants assigned to test and control group using blocks randomisation method. Patients with mild to moderate chronic periodontitis, having 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, 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.

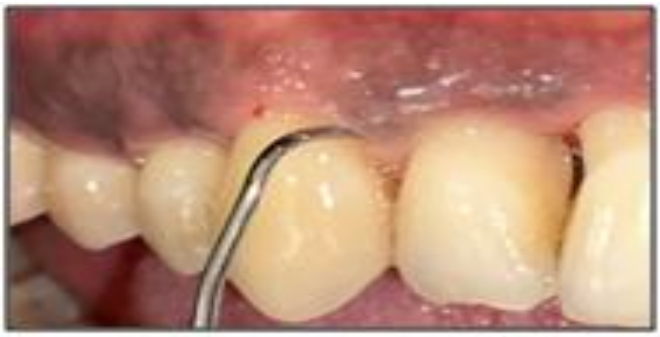
The control group (n = 10) received standard mechano therapy and placebo, whereas the study group (n = 10) received standard therapy along with Probiotic (Lactobacillus Reuteri 100 million CFU). The Microbiological parameterise presence of Porphyromonas gingivalis quantitively were analysed at

baseline and3 months. 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 dentin that is impregnated with calculus, toxins, or microorganisms.

At baseline full mouth ultrasonic scaling was done in the test and control groups and 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 sterile Gracey curette were introduced into the specific site and the plaque sample from the subgingival site is obtained. Subgingival plaque samples were transferred in separate sterile, labelled Eppendorf tubes containing 500 µL of sterilized PBS buffer and samples were immediately allowed frozen at -20ºC for further processing. In a separately labelled Eppendorf tube 21 µl of Master Mix was prepared for each samples using 5µl of Master A, 15 µl of Master B and 1 µl of Internal Control (IC) .To this Master Mix 10 µl of the extracted DNA sample was added the samples were spur down for 1 min and filled into a well of 96 welled tray of Light Cycler 480 II Real Time q PCR Machine with the help of micropipettes ,4 wells were used as controls and 1 well was used as negative control rest all the wells were filled with different prepared samples and labelling was done followed by sealing of the tray with a sealing sheet .After this the tray was spur down for 30 seconds for the removal of air bubbles . Finally, the tray was inserted into Light Cycler 480 II Real Time PCR Machine by placing it on the loading platform. Samples were run for the testing and report was generated using Light Cycler 480 software.



Group A SRP



Group A Collection of Plaque sample


Group B SRP &Collection Of Plaque sample

Probiotics Tablets

The Microbiological parameters were assessed for the presence of Porphyromonas gingivalis quantitively. The study product (Protectis-lozenges) contains Lactobacillus sporogenes 100 million CFU. 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 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 months post-operatively were evaluated. The statistical analysis was done using SPSS software Version 19

Collection of Plaque sample

Plaque sample embedded in well

Real time q PCR

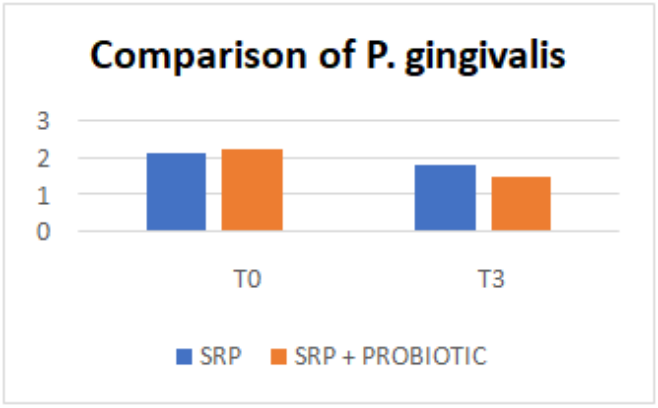
**Results**

The present randomised controlled trial was conducted to evaluate the effectiveness of SRP vs SRP with Probiotics both clinically as well as using molecular techniques ( RT-qPCR) in patients with Stage II grade B Periodontitis patients. The patients included the study comprised of 14 male and 6 female patients aged between 28-60 year. The study included two groups, where group A subjects were treated with SRP and the group B, subjects treated with SRP and Probiotics All the parameters were recorded and tabulated for both the groups at baseline, 3 months The statistical analysis was carried out using ANNOVA.

The quantitative level of P.g in Group 1 at T0and at T3 was 2.1 ng & 1.8 ng respectively and in Group II it was found to be 2.2 ng ($T_{0)}$ and 1.5 ng ($T_3$). ANOVA test yielded statistically significant results in the SRP with probiotic samples (Group II) which exhibited statistically reduction in p.g counts, which was sustained for a period of three months ($p$= 0.03).

| | Group A | Group B |
|---|---|---|
| $T_0$ | 2.1 ng | 2.2 ng |
| $T_3$ | 1.8 ng | 1.5 ng |

Quantitative analysis of P.g in both groups at baseline and after 3 months.




**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 therapeutically beneficial 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 to 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 planingwas performed 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. The presence of Porphyromonas gingivalis after 3 weeks of probiotic therapy was 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 compete for the adhesion sites thereby reduces the growth factors and nutrients available for the pathogens to sustain in the oral cavity.

**Conclusion**

One of the fastest growing fields is the therapeutic use of health-promoting bacteria for dental and general health; these microorganisms are currently available as dietary probiotics with health advantages. Clinically available evidence indicates that probiotics help to restore dental health. The findings of this pilot clinical trial reveal that Lactobacillus Reuteri has beneficial properties as an oral probiotic and decreases the prevalence of the keystone pathogen, Porphyromonas Gingivalis when used as an adjunct to Scaling and Root Planing.

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.

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