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Efficacy of Sub Gingivally Delivered Probiotic with Lactobacillus Salivarius, Lactobacillus Reuteri, Lactobacillus Plantarum as an Adjunct to Scaling and Root Planing in the Treatment of Periodontitis- A Clinical and Microbiological Study

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KEYWORDS

Periodontitis, probiotics, lactobacillus, scaling and root planing

ABSTRACT:

Introduction: Periodontal disease is a chronic microbial infection characterized by persistent inflammation, connective tissue breakdown and alveolar bone destruction. The presence of pathogenic bacteria, the absence of so-called "beneficial bacteria" and the susceptibility of the host are the main etiological factors of periodontal diseases. The administration of beneficial bacteria i.e. probiotics with antimicrobial and anti-inflammatory properties is one of several novel approaches being considered as an adjunct treatment for the management of periodontitis and may offer a low risk, inexpensive and easy-to-use treatment option.

Objectives: To evaluate the effect of sub gingivally delivered probiotics as an adjunct to scaling and root planing (SRP) on periodontal and microbiological parameters using N-benzoyl-dL-arginine-2-napthylamide (BANA) test in periodontally compromised individuals.

Methods: The study was carried out on 22 sites in different quadrants with Probing Pocket Depth (PPD) ≥ 5mm. At baseline, patients received SRP followed by subgingival delivery of probiotic to test site and placebo to control site. Plaque Index (PI), Gingival Index (GI), Sulcus Bleeding Index (SBI), Pocket Probing Depth (PPD), Relative Attachment Level (RAL) recorded at baseline, 1 month, 3 month and microbial analysis done using BANA test at baseline and 3 months.

Results: The study showed statistically significant improvement in both clinical measures as well as in the colony forming units (CFUs).

Conclusions: Probiotic strains were found effective in improving the clinical measures and could serve as a useful adjunct to SRP in periodontally compromised individuals.

1. Introduction

Periodontal disease is a chronic microbial infection characterized by persistent inflammation, connective tissue breakdown and alveolar bone destruction [1]. Both host and bacterial challenge are the key factors for the development of periodontal diseases. The presence of pathogenic bacteria, the absence of so-called "beneficial bacteria" and the susceptibility of the host are the main etiological factors of periodontal diseases [2].

Improvements in clinical parameters are seen when the levels, proportions and percentage of sites colonized by different periodontal pathogens are effectively reduced after therapy and a new microbial community with higher proportions of host compatible microorganisms is

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established [3]. However, with increasing side effects, resistance to antibiotics and desire from the general public for more 'natural' therapies, there is a need to minimize antibiotic use and develop novel treatments for oral diseases that do not involve conventional antimicrobial agents [4].

Preventive approaches based upon the restoration of the microbial ecological balance, rather than elimination of the disease associated species, have been proposed [5]. The administration of beneficial bacteria i.e. probiotics with antimicrobial and anti-inflammatory properties is one of several novel approaches being considered as an adjunct treatment for the management of periodontitis and may offer a low risk, inexpensive and easy-to-use treatment option [6]. According to World health Organization 2001, probiotics is defined as live microorganism that, when administered in adequate amounts, offer a health benefit to the host [7]. Evidence documented the potential health benefits of probiotics when used systemically in periodontitis patients [8-9] but investigations regarding their use as local delivery agent at the site of infection are limited [10-11].

Moreover, to the best of our knowledge there is no published data investigating the local delivery of probiotics with Lactobacillus (L) salivarius, Lactobacillus plantarum and Lactobacillus reuteri in the treatment of periodontally compromised individuals.

2. Objectives

To evaluate the effect of sub gingivally delivered probiotics as an adjunct to scaling and root planing (SRP) on periodontal and microbiological parameters using N-benzoyl-dL-arginine-2-napthylamide (BANA) test in periodontally compromised individuals.

3. Methods

A total of 22 sites were selected amongst the walk-in patients diagnosed with periodontal disease from the Outpatient Department of Periodontology, School of Dental Sciences, Sharda University, Greater Noida. The ethical clearance from Institutional Ethics Committee No. SU/SMS&R/76-A/2018/123, Sharda University was taken. The nature and outcome of the study were explained to the patients and a written consent form was obtained. For the purpose of standardization, two pockets with 5-7 mm pocket depth in two different quadrants were chosen as the test and control sites. Randomization and allocation done using coin toss method into test and control sites. In control sites, SRP was performed and placebo gel was placed whereas in test sites, SRP was followed by placement of probiotic gel **[Figure 1].**

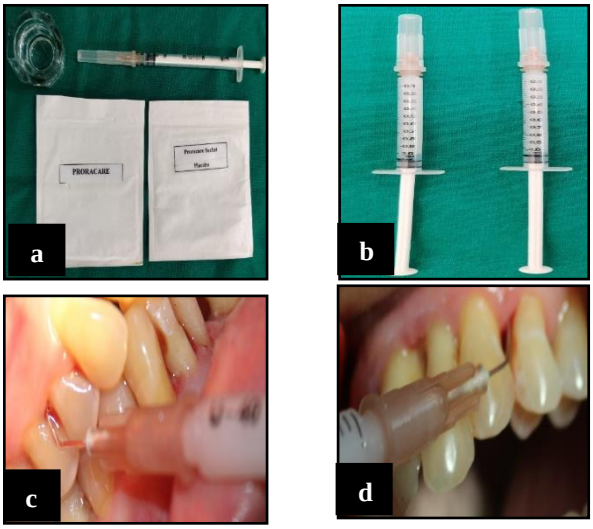

Figure 1. Showing gel formulation and subgingival delivery in periodontal pockets in test and control groups (a) Probiotic and placebo sachets (b) Tuberculin syringe loaded with probiotic and placebo gel (c) Probiotic gel delivered sub gingivally into periodontal pocket in test group (d) Placebo gel delivered sub gingivally into periodontal pocket in control group.

Systemically healthy patients of age ≥ 30 years having probing pocket depth (PPD) ≥ 5mm and clinical attachment level (CAL) ≥ 3 mm in at least one site each in any of the two contralateral quadrants with radiographic evidence of bone loss were included in the study. Smokers, medically compromised patients, pregnant or lactating women, patients who have undergone periodontal surgery, restorative procedure and tooth extraction adjacent to either of test teeth area in the previous 3 months, patients already on probiotic therapy or antibiotic therapy in the 6-month period prior to the study were not included. PPD and Relative attachment level (RAL) were recorded using a University of North Carolina no.15 (UNC-15) colour coded periodontal probe at six sites of the tooth surface. PPD was measured to the nearest millimetre from the gingival margin to the base of the clinical pocket. Prefabricated stents were prepared for assessment of RAL and was recorded at baseline, 1 month and 3 months after treatment **[Figure 2]**. Site specific gingival index (GI),

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plaque index (PI), sulcus bleeding index (SBI), PPD were measured at baseline, 1 month, 3 months after treatment.

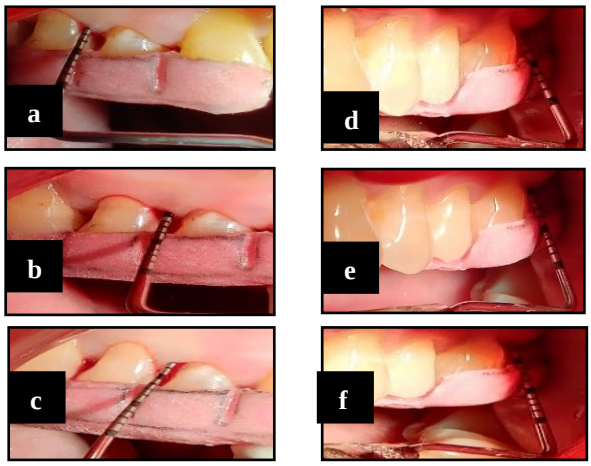

Figure 2: Relative attachment level in test site at: (a) baseline (b) 1 month (c) 3 months; Relative attachment level in control site at: (d) baseline (e) 1 month (f) 3 months

Formulation of probiotic mixture: A combination of three probiotic organisms was used which included Lactobacillus salivarius, Lactobacillus plantarum and Lactobacillus reuteri with 0.5×10^9 Colony Forming Units (CFUs) per sachet. These organisms were supplied by Unique Biotech laboratories, Hyderabad, in sachets form. The contents of sachets were mixed with distilled water to prepare a gel that was then inserted into the test sites. **Microbial sampling and analysis:** The Benzoyl-DL-arginine-2-napthylamide (BANA) chair side test is used for detecting red complex organisms associated with periodontal disease (Treponema denticola, Porphyromonas gingivalis and Bacteroides forsythus) in subgingival plaque samples. BANA a colourless substrate, it releases β- naphthylamide, which turns orange red when a drop of fast garnet is added to the solution. The degree of colour change assessed as follows:

No colour change: Zero or negative, $<10^4$ CFU

Faint blue to black colour: One or weak positive, 10^4 - 10^5CFU

Distinct blue-black colour: Two or strong positive, $>10^6$CFU

The plaque sample was collected from each patient at baseline and 3 months. Sites with deepest PPD were selected for plaque collection. The site was isolated with cotton rolls and air dried. A sterile Gracey curette was gently inserted into the pocket along the root surface, and care is taken to avoid injury to the gingival tissue and avoid bleeding in that area. Each plaque sample was deposited on the BANA impregnated filter strip, which was at the lower border of the strip and upper reagent strip containing Evans black dye, which was activated by wetting the strip with water or saline. The lower strip was then folded over the upper strip where it would react with Evans black dye showing the colour change. The two strips were held together and incubated in a heating block (incubator) for 15 min at about 55°C after which the card is removed, and the degree of colour change was assessed.

STATISTICAL ANALYSIS The sample size was calculated using the n Master 2.0 software. The power of the study was taken to be 80% and Confidence Interval (C.I) of 95% was taken. The sample size calculation was done using the change in PPD. Descriptive statistics was performed by calculating mean, standard deviation, frequencies and percentages for the Continuous variables. Categorical variables where be summarized as frequencies and percentages. Shapiro Wilk test used to check whether the continuous variables were following normal distribution or not. Inferential statistics done using the chi-square test for the categorical variables whereas the unpaired t-test used for the comparison of continuous variables between the 2 groups. Repeated measures ANOVA test with post-hoc Bonferroni test for the comparison of the mean values over a period of time. The suitable non-parametric test used where the data does not follow the normal distribution. Level of statistical significance was set at p-value less than or equal to 0.05.

4. Results

The present study was a double-blinded, split mouth randomized controlled clinical trial conducted to evaluate clinical and microbiological efficacy of sub gingivally delivered probiotic with L. salivarius, L. reuteri, L. plantarum as an adjunct to scaling and root planing (SRP) in the management of periodontally compromised individuals. The intragroup comparison of mean PI & GI at both test and control sites showed a statistically significant reduction between baseline, 1 month and 3 months ($p < 0.001$) whereas in the intergroup

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comparison, there was no statistically significant difference in mean PI at Baseline,1 month and 3 months ($p < 0.001$) between test sites and control sites. The intragroup comparison of mean SBI, PPD & RAL at both test sites and control sites showed a statistically significant difference between baseline, 1 month and 3 months ($p<0.001$)**,** however**,** in intergroup comparison the mean SBI, PPD & RAL showed statistically significant reduction among the test sites when compared to control sites. The intragroup comparison in terms of degree of colour change at test sites had shown statistically significant difference from baseline to 3 months ($p=0.005$). The intergroup comparison in terms of degree of colour change in test sites and control sites showed statistically significant difference from baseline and 3 months in test sites when compared to control sites. The BANA score at baseline and 3 months was compared between test site and control site using chi-square test, showed faint blue colour at 3 months which was significantly more among control sites. The intragroup comparison in terms of CFUs at test sites showed statistically significant difference from baseline to 3 months. The intergroup comparison in terms of CFUs at test sites showed statistically significant difference when compared to control sites ($p=0.005$). BANA score showed that >10^4 CFUs was significantly more among control site at 3 months**.**

5. Discussion

Periodontitis is known to have multifactorial aetiology involving interplay between environmental, host and microbial factors (12). The current concept of aetiology considers three groups of factors which determine whether periodontal destruction occurs by a susceptible host, the presence of pathogenic species, and the absence of beneficial bacteria (13). Several studies have been done using different strains of probiotics and have demonstrated improved clinical and microbial parameters in periodontitis patients (14-16). Commonly used probiotics in periodontitis patients are L. salivarius, L. reuteri, L. rhamnosus, L. acidophilus, L. plantarum, Saccharomyces boulardii and several other strains including Bifidobacterium. It has been documented that "no single probiotic bacterium exhibits all the properties, at least not to the extent that it could be a remedy for prevention therapy of all types of diseases" therefore probiotics strains are often used in combination with each other, in order to increase the number of beneficial effects (17). To best of our knowledge this is the first study evaluating effectiveness of combination of three probiotic strains (L. salivarius, L. reuteri, L. plantarum) on clinical parameters and microbiological parameters using BANA. In accordance to present study, past research has evaluated the effects of L. reuteri (Prodentis lozenges) alone and in combination with SRP in a double blind, randomized, placebo-controlled clinical trial of volunteers with chronic periodontitis where result have shown reduction in all clinical parameters PI, GI, and GBI significantly (18). Evidence supports that periodontitis patients are benefited in terms of reduction in PPD, SBI, RAL when treated with L. brevis and L. plantarum strains, applied into periodontal pockets as gel and lozenges, along with SRP (19-21). Present study has evaluated the above result that adjunctive use of probiotics has improved all clinical parameters. In agreement with earlier studies, combination of probiotics (L. salivarius, L. reuteri, L. plantarum) used in our study shown to suppress pathogen growth through the release of a variety of antimicrobial factors like defensins, bacteriocins, hydrogen peroxide, nitric oxide, and short chain fatty acids (SCFA) such as lactic and acetic acids, which reduce the pH of the lumen (22-23). This reduction in bacterial load, pro-inflammatory cytokines and release of antimicrobial agents might have resulted in improved clinical parameters in test group as compared to control group. Probiotics combat dysbiosis by competitive inhibition of periodontal pathogens, and thereby reducing the overall immunogenicity of the oral microbiota. Secondly, modulate active disease-associated immune/inflammatory pathways to reduce the destructive inflammation of periodontitis and lead to immune homeostasis that could be maintained by the host in the long term (24).

This study has also showed the anticipated mechanisms of probiotics (L. salivarius L. reuteri, L. plantarum) following subgingival delivery into periodontal pockets indicating that the positive effects of probiotics on oral health are based on a unique mechanism of maintaining ecological balance, competitive inhibition, downregulating inflammatory cytokines and immunomodulation which are stated in previous studies (25-26). It is reported that L. reuteri forms reuterin (3-hydroxipropionaldehyd), which induces oxidative stress in cells, has ability to compete with pathogens for epithelial cell adhesion, a reduction in the production of

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proinflammatory cytokines (TNF-α, IL-1β, IL-17), a reduction in MMP-8 expression, which is the main collagenase involved in periodontitis, and an increase of TIMP-1, which is a modulating factor of MMP activity (24). Microbial assessment was done with Benzoyl-DL-arginine-2-napthylamide (BANA), which qualitatively assesses the red complex bacteria, by means of an enzymatic reaction, represented as a colour change on the strip. Present study resulted statistically significant reduction in BANA scores observed in the test group as compared to control group, this is due to the decrease in microbial load attributed to combined effects of SRP and probiotics as well as reinforcement of oral hygiene measures which is the standard treatment procedure in disrupting the subgingival microbial environment. Our study supports the hypothesis of guided pocket recolonization, wherein the beneficial bacteria populate the sulcus and prevent the recolonization of periodontal pathogens by means of competitive inhibition (27). In addition, present study has also evaluated the efficacy of probiotic strains and shown that the combination of probiotic strains (L. salivarius L. reuteri, L. plantarum) seemed to be antagonistic to P. gingivalis, by producing antimicrobial substances such as reuterin (L. reuteri), bacteriocins (L. salivarius) and plantarin (L. plantarum) and eliminate them from their binding sites. Although the aim and objectives of present study were met, fewer limitations were also noted i.e. study has smaller sample size, changes were assessed at 1 month and 3 months only, only single dose of probiotic gel was used in each patient due to COVID pandemic. Further longitudinal studies are required to assess the effectiveness of probiotics with L. salivarius, L. reuteri, L. plantarum strains for the management of periodontally compromised individuals.

6. Conclusion

Addressing the growing concern over multidrug resistance through antibiotic usage, there has been renewed interest in bacterial replacement therapy in recreating healthy microbial environment. Future randomized controlled clinical trials can be conducted using larger sample size of longer duration involving multiple doses along with use of systemic administration of probiotics to evaluate the effectiveness of probiotics as an adjunct to scaling and root planing. Our study gives an insight towards the non-surgical approach in the treatment of moderate to severe periodontal pockets rather than surgical approach which can be sometimes deterrent to the patients as well as to the clinician in the treatment of periodontal disease. Multicentred research trials should be conducted to evaluate the effectiveness of probiotic strains in patients with gingivitis, periodontitis, aggressive periodontitis, in smokers with periodontitis, peri-implant mucositis and peri-implantitis.

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