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Evaluation of Efficacy of Probiotic Lozenges as an Adjunct to Scaling and Polishing in the Treatment of Moderate Gingivitis – A Clinical and Microbiological Study.

Dr Abdul Aziz[1], Dr. Vishakha Patil[2], Dr. Pooja Pharne[3], Dr. Yogesh Khadtare[4], Dr. Rajeeta Joseph[5], Dr. Dilip Magdum.

[1]Al Abeer Medical Center, Jeddah.
[2]Bharati Vidyapeeth Deemed to be University Dental College and Hospital, Pune.
[3]Bharati Vidyapeeth Deemed to be University Dental College and Hospital, Pune.
[4]Bharati Vidyapeeth Deemed to be University Dental College and Hospital, Pune.
[5]Bharati Vidyapeeth Deemed to be University Dental College and Hospital, Pune.
[6]Bharati Vidyapeeth Deemed to be University Dental College and Hospital, Sangli.

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KEYWORDS

Probiotics, Gingivitis, *Actinomyces viscosus*, Synbiotics, Lozenges.

ABSTRACT:

Introduction: In Periodontal disease, both host and bacterial challenge are the main etiological factors for tissue destruction The presence of pathogenic bacteria and the absence of beneficial bacteria along with susceptibility of the host are the key factors responsible for periodontal involvement. When healthy flora is maintained, it may inhibit colonization by pathogenic bacteria, thus protecting the host from the development of periodontitis. Probiotics (living microorganisms) confer health benefits on the host when administered in sufficient doses

Objectives: To evaluate and compare the efficacy of Probiotic lozenges as an adjunct to Scaling and Polishing with Scaling and Polishing alone in Chronic moderate Gingivitis subjects. Assessment and comparison of Plaque index, Gingival index and C. Actinomyces viscous microbial profile before and after using probiotic lozenges as an adjunct to scaling and polishing with scaling and polishing alone in gingivitis subjects.

Methods: 50 participants recruited were divided into Control and Probiotic group. Both groups underwent scaling and polishing, while Group B was advised Probiotic lozenges twice a day for two weeks.

Results: Statistically highly significant reduction was seen in Mean Plaque index and Mean Gingival index in both the groups from baseline to 2 weeks post-treatment. Statistically there was no significant difference when both the groups were compared. The microbial profile of Actinomyces Viscosus showed statistically highly significant reduction at 2 weeks with Probiotic lozenges group as compared to Control group.

Conclusions: Probiotic lozenges may be used as an adjunct to non-surgical therapy in the treatment of Gingivitis and thus help halt the growth of microorganisms and prevent its progression to periodontitis.

1. Introduction

Periodontal disease describes a group of related inflammatory diseases affecting the supporting structures of the tooth. It can broadly be classified into gingivitis and periodontitis. Bacterial plaque is proved to be the principal causative factor in gingival and periodontal diseases (Löe H 1965) [1]. Gingivitis is characterized by inflammation limited to the gingiva. If proper treatment

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and care is taken in its initial stage, it can prevent extension of this inflammatory process to the supporting periodontal tissues. Various Plaque control measures (mechanical and chemical) are effective. However, their effectiveness depend on skills and techniques of carrying out these procedures. Also difficulty in maintaining plaque control in difficult to reach areas (interproximal) further necessitates the use of an adjunct to mechanical plaque control (Fine DH 1995) [2]. The periodontium has recently been suggested as a relevant target for probiotic applications. Probiotics, the "friendly bacteria" or "good bacteria" which adhere to dental tissues as a part of biofilm, provide a protective lining for oral tissues against these oral diseases (Comelli EM 2002) [3]. Such a friendly biofilm may keep bacterial pathogens away from oral tissues by filling up space which could have served as a niche for pathogens in future.

The term probiotics is defined by "The International Scientific Association" as "live microorganisms which when administered in adequate amounts, confer a health benefit on the host" (Colombo M 2018) [4]. The most commonly used and studied probiotics are lactic acid bacteria, in particular Lactobacillus spp (Abdel Hamid AG 2019) [5]. and Bifidobacterium spp (Makras L 2006) [6]. Synbiotic is a novel approach where probiotics and prebiotics are combined in an attempt to obtain synergistic effects and faster colonization of useful bacteria. Synbiotic therapy containing probiotics such as Lactobacillus sporogens 50 million, Streptococcus faecalis T-110JPC 30 million, Clostridium butyrium TO-A 2 million and Bacillus mesentericus TO-A JPC 1 million is found to be active against wide range of gram positive bacteria and few gram negative bacteria (Malathi K 2019)[7].

Considering all the above claims and facts, this study was planned to test the anti-plaque and anti-inflammatory properties of Probiotic in the form of lozenges which may prove to be cost effective and biologically acceptable.

2. Methods

The study was carried out at Post Graduate clinic of Department of Periodontology. The subjects were recruited from the Out Patient Department of Periodontology at Bharati Vidyapeeth (Deemed to be) University Dental College and Hospital, Pune. A total of 50 Subjects with age ranging from 18 to 55 years having chronic moderate gingivitis, with no history of any antibiotic therapy since one month, and history of non consumption of any Probiotic supplements were included in the study. Pregnant women and subjects with history of any systemic diseases were excluded. All subjects were given written and verbal instructions about the study and written informed consent was obtained from each subject. Consent forms were provided and subjects willing to participate were included. Participants were assessed for plaque and gingivitis score prior to commencement of study and those with moderate gingivitis were recruited for the study. The indices included for the study were Plaque Index (Loe H 1967)[8]. and Gingival Index (Löe H 1963)[9]. Participants were equally divided into two groups. Group A (Control group) and Group B (Probiotic group).

Plaque and Gingivitis index readings were considered as baseline readings. Clinical data was recorded in a special proforma. Pre-treatment plaque samples were also collected and microbial profile assay was carried out for Actinomyces viscosus (Colony forming unit count) using Agar plates.

Microbial profile Assay:

It was carried out using Blood Agar plates. Media Used was NACBA – Nalixidic acid Colistin selective medium. Agar plates were brought to room temperature before use. Inoculum preparation was done by using a loop or swab. Turbidity was adjusted visually with broth to that of a 0.5 McFarland turbidity standard that has been vortexed. Alternatively, suspension was standardized with a photometric device. Inoculation of Agar plate was done within 15 min of adjusting the inoculum to a 0.5 McFarland turbidity standard (Swenson JM 1984) [10], a sterile cotton swab was dipped into the inoculum and rotated against the wall of the tube above the liquid to remove excess inoculum. Entire surface of agar plate was swabbed three times, and plates were rotated approximately 60º between streaking to ensure even distribution. Hitting sides of petri plate and creating aerosols was avoided. Inoculated plate was allowed to stand for at least 3 minutes but no longer than 15 min before making wells. A hollow tube of 5mm diameter was heated and pressed on inoculated Agar plate and removed immediately by making a well in the plate (Schwalbe R 2007) [11]. Likewise, five wells were made on each plate. 2.75µl, 50 µl, 25 µl, 10 µl and 5 µl of

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compound was added into the respective wells on each plate. Plates were incubated within 15 min of compound application and were incubated and stacked. Plates were incubated for 48-72 hours at 37 ºC in Anaerobic jar. Plates were read only if the lawn of growth was confluent or nearly confluent. Diameter of inhibition zone was measured to nearest whole millimetre by holding the measuring device. Then after completion of incubation, the plates were removed and colony was noted. Characters of the required organism and also the colony count were done for quantification. Actinomyces viscosus gave small, white opaque colonies (Valour F 2014) [12]. These organisms were confirmed by Gram staining and key biochemical.

All the participants of both the groups were subjected to scaling and polishing. However, Group B participants were advised to use Probiotic lozenges twice a day (after brushing) for up to two weeks.

Experimental Lozenges:

Probiotic lozenges were obtained from Tablets (India) Limited, Marshal Road, Chennai. Each Probiotic lozenges contain Streptococcus faecalis T-110 JPC - 30 million, Clostridium butyricum TO-A- 2 million, Bacillus mesentericus TO-A - 1million, Lactobacillus sporogenes- 50 million with orange flavor. In Probiotic lozenges, a unique tooth friendly, non-cariogenic, sweet tasting isomalt was used as a base. This sugar free probiotic lozenge is developed, based on unique technology transfer of TOA, Pharmaceuticals, Japan.

Participants were recalled after two weeks and recording of same indices and collection of plaque samples for assessment of microbial profile was carried out.

After recording of all the data, it was arranged in master chart and subjected to statistical analysis.

Statistical analysis:

Descriptive and inferential statistical analysis was carried out in the study. Results on continuous measurement were presented on mean (SD) and results on categorical measurement were presented in number (%). Level of significance was fixed at p=0.05 and any value less than or equal to 0.05 was considered to be statistically significant. Student "t" tests (two tailed, paired and unpaired) were used to find the significance of study parameters on continuous scale between two groups. The statistical software IBM SPSS statistics 20.0 (IBM Corporation, Armonk, NY, USA) was used for the analyses of the data and Microsoft word and Excel were used to generate graphs, tables etc.

3. Results

Intra-group comparison of Mean Plaque Index score, Gingival Index score and Colony forming unit count for both the groups (Control group as well as Probiotic group) showed statistically significant difference ($p < 0.001$) at baseline and two weeks (Table 1 and 2). There was significant reduction in these parameters in both the groups at 2 weeks.

Inter-group comparison of Mean difference of Plaque Index score, Gingival Index score for both the groups (Control group as well as Probiotic group) (Table 3) showed statistically no significant difference ($p \geq 0.05$) at baseline. At 2 weeks, values of above parameters showed statistically non-significant mean difference ($p \geq 0.05$) from baseline to 2 weeks, indicating that both the groups were equally effective at 2 weeks. No group was superior or inferior to each other. However, when mean difference was considered for both the groups for Actinomyces Viscosus colony forming unit count from baseline to 2 weeks after scaling and polishing, it was noticed that there was statistically highly significant mean difference ($p < 0.001$) from baseline to 2 weeks. The mean difference was found to be more with Probiotic group as compared to that of control group.

4. Discussion

Lilly and Stillwell in 1965 (Lilly DM 1965) [13] put forth the term "probiotic" as "growth-promoting factors produced by microorganisms." In 2002, WHO along with Food and Agriculture Organization of the United States (FAO) (Hill C 2014) [14] stated the term probiotics as "living microorganisms that can have a beneficial effect on the host when taken in sufficient doses." Probiotics usually use naturally occurring microorganisms and confer health benefits in cases of periodontitis too, when given in an adequate amount. These genera of bacteria are capable of halting, altering, or delaying periodontitis. Probiotics have been used mostly to reduce plaque accumulation, to modify colonization of anaerobic microorganism, reduce the depth of the pocket and to improve the attachment level (Chatterjee A 2011)[15].

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Probiotics, can affect the periodontium in different ways. It plays important role in immunomodulation (Erickson KL 2000)[16]. It stimulates antigen presenting cells (mostly dendritic cells) resulting in expression of Th1 (T-helper cell 1) or Th2 (T-helper cell 2) response, thus modulating immune system. Innate immunity is also enhanced. Th1 response helps phagocytosis of Intracellular pathogens, whereas Th2 response takes care of extracellular pathogens. Probiotics can also mimic response similar to a pathogen but without periodontal destruction. Glycoprotein – carbohydrate cell surface interaction is also mediated by inter species interactions (Grimaudo NJ 1997)[17]. Probiotics help decrease pro inflammatory cytokines in gingival crevicular fluid if prescribed with chewing gum containing Lactobacillus reutri (Twetman S 2009)[18].

Probiotic microorganisms such as Lactobacillus casei, Lactobacillus bulgaricus, Lactobacillus rhamnosus, Lactobacillus acidophilus co-aggregate with periodontal pathogen such as Fusobacterium nucleatum. Probiotic Lactobacillus rhamnosus, and Lactobacillus paracasei have strong binding activity to primary pellicle. Lactobacillus rhamnosus, Lactobacillus caseishirota, Lactobacillus casei ATCC 11578 prevent adherence of bacteria to salivary pellicle by altering its composition. Lactobacillus is a strongest inhibitor of Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis and Prevotella intermedia thus altering its aggregation(Chatterjee A 2011) [15]. Lactobacillus salivaris and Lactobacillus gasseri show strong inhibition of periopathogenic bacteria (Matsuoka T 2006)[19]. Secretion of bacteriocins by Lactobacillus reutri, e.g., reutrin (Cleusix V 2007)[20] and reutericyclin (Ganzle MG 2000) [21] inhibits growth of pathogens and has high affinity for host tissue and has anti- inflammatory effect by inhibition of proinflamatory mediators (Grudianov AI 2002) [22].

Another important mechanism seen with probiotics is apoptosis (programmed cell death by the innate immune system). Probiotics stimulate apoptosis of tumor cells through end product formation (Iyer C 2008)[23]. It inhibits apoptosis of mucosal cells (Yan F 2002)[24] Probiotic mixture protects the epithelial barrier by maintaining tight junction protein expression thus preventing apoptosis.

Probiotics, thus have proper scope in the field of treatment of periodontitis. Therefore the use of probiotic lozenges can be beneficial an adjunct to scaling and root planning for its wide range of effects.

The results obtained in our study indicate that the Synbiotic (Probiotic) lozenges (Streptococcus faecalis T-110 JPC - 30 million, Clostridium butyricium TO-A- 2 million, Bacillus mesentericus TO-A - 1million, Lactobacillus sporogenes- 50 million with orange flavor) used in this study as an adjunct to scaling and polishing for a period of two weeks were strongly effective in reducing the plaque score and gingivitis score from baseline to two weeks. It was also beneficial in adequately reducing the Actinomyces viscosus colony forming unit count at two weeks from baseline. Both control as well as probiotic groups were equally effective at 2 weeks. However, when mean difference was considered for both the groups for Actinomyces Viscosus colony forming unit count from baseline to 2 weeks, probiotic group showed drastic improvement in these counts at two weeks.

Similar results have been quoted by various studies in the literature. Teughels W (2013)[25], evaluated the effects of Lactobacillus reuteri containing probiotic lozenges as an adjunct to scaling and root planing (SRP) in thirty chronic periodontitis patients at baseline, 3, 6, 9 and 12 weeks after therapy. Plaque index scores, Gingival index score and Actinomyces viscosus colony forming unit count at the end of twelve weeks (Table 2) was significantly reduced. Also in a randomised, placebo-controlled, double blind study by Krasse and colleagues in year 2006[26] L.reuteri supplemented probiotics (two different probiotic formulations (LR-1 and LR-2)) given for a period of 2 weeks in moderate to severe form of gingivitis cases was efficacious in reducing Plaque and Gingival inflammation on 14th day. K. Noordin, S.Kamin in year 2007[27] found probiotic mouthrinse containing nisin to be effective on dental plaque and gingival inflammation in humans. Participants used placebo mouthrinse (for two weeks), which was followed by a washout period of 4 weeks. They then used probiotic mouth rinse for a further duration of two weeks. Study by Shivani Dhawan and Rajan Dhawan in year 2013[28] also reported statistically significant reduction in Streptococcus mutans levels at the completion (T2) of medication and at a period of further 2 weeks (T3). The result of our study is however contradictory to the results achieved in the study carried out by Hallstrom H. et al in year 2013[29].

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They asked participants to use lozenges containing L reuteri (ATCC55730 and ATCC PTA5289) twice a day. They concluded that its daily intake did not seem to significantly affect the plaque accumulation during experimental gingivitis.

Thus, it can be concluded that probiotic lozenges are effective when used as an adjunct to scaling and polishing in gingivitis cases. It can routinely be indicated for periodontitis patients also for its added benefits such as it is cost effective, non-allergic, non-pathogenic, and commercially easily available as well as safe. It can, at its best reduce the plaque formation and thus prevent further gingival and periodontal problems. Hence, proving it to be a best option in such cases.

It can thus be concluded that Gingival and periodontal diseases have been affecting the majority of population across the world. Several types of accretions occurring on the teeth are associated with periodontal disease in one way or the other. Amongst them dental plaque has imposed a real challenge. As bacterial plaque is the principal causative factor in gingival and periodontal diseases, the most rational methodology for the prevention of periodontal diseases would be regular, effective removal of plaque and prevention of its formation. Regular mechanical means of plaque control include brushing, flossing, use of interdental cleansing aids and oral prophylaxis. However, most of the people experience difficulty in maintaining it particularly at interproximal sites. Use of probiotic lozenges has shown to have promising results. Present study also has proved its advantageous effects in subjects with chronic generalized gingivitis. It can thus be concluded that use of Probiotic lozenges for a period of two weeks is also enough to get desired results.

Future Directions: To elucidate the use of Probiotic lozenges as an adjunct to Scaling and Polishing, further studies with larger sample size should be carried out. Microbiologic evaluation can be done for more specific periodontal pathogens. Much more scientific developments are needed to have a better understanding of these microorganisms in order to broaden their potential applications.

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