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Original Article

Clinical effect of pro-biotic containing Bacillus coagulans on plaque induced gingivitis : A randomised clinical pilot study.

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Abstract :

Objective : To evaluate the clinical effect of *Bacillus coagulans* on plaque induced gingivitis.

Method : Thirty subjects with plaque induced were enrolled in the study. At baseline, Gingival Index (GI) and Plaque Index (PI) were assessed. Saliva samples were collected for glutathione peroxidise (GPx) activity analysis and to determine load of lactobacilli. Subsequently participants were randomly provided with chewable tablets to be consumed 3 X daily for 3 month containing 100 million colony forming units (CFU)/tablet of B. coagulans or without B. coagulans (placebo). After3 months, recording of GI, PI and saliva sampling were repeated.

Result : At baseline, mean GI, and mean PI did not differ significantly between both groups. At re-evaluation, mean GI, and bleeding on probing of the probiotic group were both significantly ($p < 0.0001$) lower than in the placebo group. Mean PI level did not differ significantly between the groups. In the probiotic group, mean glutathione peroxide activity (Gpx)was significantly ($p < 0.02$) lower than in the placebo group at re-evaluation.

Conclusion : The consumption of probiotic containing Bacillus coagulans seems to modulate inflammatory response in plaque induced gingivitis.

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Introduction

Plaque induced gingivitis is a frequent clinical finding, with a prevalence of over 90% in the general population. Plaque induced gingivitis is reversible, but may progress to periodontitis, in susceptible patients, if left untreated. Professionally administered plaque removal followed by reinstitution of oral hygiene is indispensable in maintaining periodontal health.[1]

However, failure to completely resolve gingival inflammation remains a problem as plaque removal may not significantly reverse the overgrowth of sub-gingival flora. It has been hypothesised that residual inflammation following plaque removal, aids the stabilization of sub-gingival flora composition, even in the absence of plaque or periodontal pockets. The persistence of specific pathogenic phyla even after oral hygiene measures may result in progression of periodontal disease. [2]Hence, in order to enhance the clinical outcome of plaque removal, several adjunctive treatments such as antiseptics, local and systemic antibiotics, photodynamic therapy have been proposed to reduce the microbial flora. However, none of these adjunctive treatments have shown superior efficacy to scaling and root planing alone. [3]

Bacterio therapy, is a promising alternative adjunctive treatment method to combat inflammation. Probiotics are one of these new agents. Probiotics are harmless living micro-organisms which, when administered in adequate amounts, confer a health benefit to the host by suppressing/displacing endogenous and exogenous pathogens and by modulating the immune system towards an anti-inflammatory response. [4]

To date, a number of studies have evaluated the adjunctive efficacy of probiotics containing different Lactobacillus and Bifidobacterial strains for the treatment of plaque induced

gingivitis with conventional scaling and root planing, with positive results. In addition, there are other genera of bacteria like *Bacillus coagulans* that have probiotic effect and may have a positive effect in the management of periodontal disease. [4,5]

Bacillus coagulans, earlier known as Lactobacillus sporogenes, is a gram positive, lactic acid producing, and spore forming bacteria, which is non-pathogenic and safe for human consumption. Due its spore forming characteristics these bacteria have the potential to survive industrial processing. It is reported to support a healthy balance of microflora in the intestinal environment. To the investigator's knowledge, there are no controlled studies evaluating their role in the prevention and treatment of gingivitis. [6, 7, 8]

Since, the effects of probiotics are strain-, dosage- and mode of application-dependent,[9] the aim of the present pilot study, was to evaluate the clinical efficacy of probiotic containing *Bacillus coagulans* in a chewing tablet form in the treatment of plaque induced gingivitis.

Materials and Method

Study Population

A double-blind, placebo-controlled, prospective study was designed.(Table I) The study protocol was approved by the institutional ethical committee and written consent was obtained from patients before enrolling them for the study.

Subjects in age group of 18-50 years, diagnosed with gingivitis, based on the gingival index score (Loe and Silness, 1963)[10], in the Department of Periodontics of A. B. Shetty Memorial Institute of Dental Sciences, Deralakatte, Mangalore, were included. Patients were randomly assigned to probiotic group (test) or the placebo group (control), with 15 patients in each group. The second author (NS) was the only person aware of treatment given to the patient's, and coding was not unblinded until the end of the study. The clinical examiner (MJ) and the patients were blinded.

The exclusion criteria were: (i) patients receiving any kind of local or systemic decontamination treatment of the oral cavity in the last 3 months, or periodontal treatment in the last 6 months; (ii) uncontrolled periodontal disease; (iii) patients with systemic disorders capable of influencing the treatment results; (iv) smokers; (v) incomplete protocols due to a lack of patient cooperation; (vi) failure to provide informed consent to participate in the study ; (vii) Patients on antibiotic therapy.;(viii) Subjects using probiotic supplements; x) Pregnant, lactating and women in their menstrual phase. The inclusion criteria were: (i)) Subjects with minimum complement of 20 teeth; (ii) Systemically healthy subjects with moderate to severe gingivitis.

Probiotic Product

The study product (probiotic) *Bacillus coagulans*, which was available as free flowing, greyish white powder (Unique Biotech Limited, Hyderabad, India) was manufactured in the form of chewable tablets at Srinivas College of Pharmacy, Valachil. Each probiotic tablet contained 100 million colony forming units (CFU)/tablet. It was tested against a placebo tablet containing starch (150mg/tablet), identical in appearance with the probiotic. The dose was 3 tablets to be taken orally every day throughout the test period. Subjects were instructed to place the tablet in the oral cavity for few minutes, allowing them to dissolve.

Clinical parameters

All clinical measurements were obtained from Ram fjord's six teeth (16, 21, 24, 36, 41& 44 in the FDI two-digit notation system) [11] in all subjects at each visit and considered representative of the whole dentition. Bleeding on probing (BOP)[12], Gingival Index (GI) [10] and Plaque Index (PI) [13]were recorded.

The study was performed over a 3week period. Test group subjects and control group subjects were given probiotic tablets and placebo tablets respectively thrice daily for 3 weeks as per the above-mentioned instructions. Subjects were instructed not to change their oral hygiene regimens. No oral hygiene instructions were given under the experimental period. Clinical parameters were recorded and saliva samples were collected from all subjects on days 0 (baseline; BL) and 22 (3 weeks; 3W).

Collection of Saliva

Un stimulated whole saliva samples were obtained by expectoration. Subjects were asked to spit approximately 3.0 ml of saliva into sterile bottles. Saliva samples were collected between 09:00 and 12:00 hours. Samples were stored at 4°C until use and then delivered to the laboratory for analysis.

Glutathione Peroxidise Activity

The collected saliva samples (1ml) were centrifuged at 2000 rpm for 5 minutes and the supernatant was stored in clean Eppendorf tubes in the freezer at -20°C until analysis was done. Glutathione peroxidise activity in the saliva was determined by using UV Spectrophotometer at 412 nm. The enzyme activity was expressed as µg of glutathione (GSH) consumed/min/mg protein.

Microbiological Analysis

The saliva samples (1ml) were streaked onto Rogosa SL HiVeg™ Agar media and incubated at 37°C for 24 to 48 hours. The colonies of lactobacilli were confirmed by Gram staining and colony morphology. The load of lactobacilli in the saliva was determined by microbial assay.

Statistical Analysis

The obtained data was analysed using unpaired Student's t-test, to compare the before and after values. A p-value of < 0.001 was considered to be statistically significant (s) and p-value of >0.05 was considered not significant (ns).

Results

30 patients were included in the study and randomized in two groups of 15 patients (test or placebo). All patients attended all the programmed visits. Results of the study are summarised in table II

Clinical outcome

A significant difference was detected between the groups for BOP ($p<0.0001$). A statistically significant decrease in BOP in the probiotic group subjects was observed from baseline till the end of the study ($p < 0.0001$), while no significant difference was found in BOP in the placebo group subjects ($p > 0.05$).

For GI, a statistically significant decrease was observed in the Probiotic group subjects after the experimental period ($p<0.0001$), while no statistically significant difference was seen in placebo group subjects after the study period ($p>0.05$). [Table II]

For PI, no statistically significant difference was observed over the study period in both the Probiotic and Placebo groups ($p>0.05$).

Microbiological outcomes in saliva samples

There was no statistically significant difference in the colony count of Lactobacilli in saliva in both the probiotic and placebo groups during the study period. (Data not shown)

Glutathione peroxide (GPx) activity

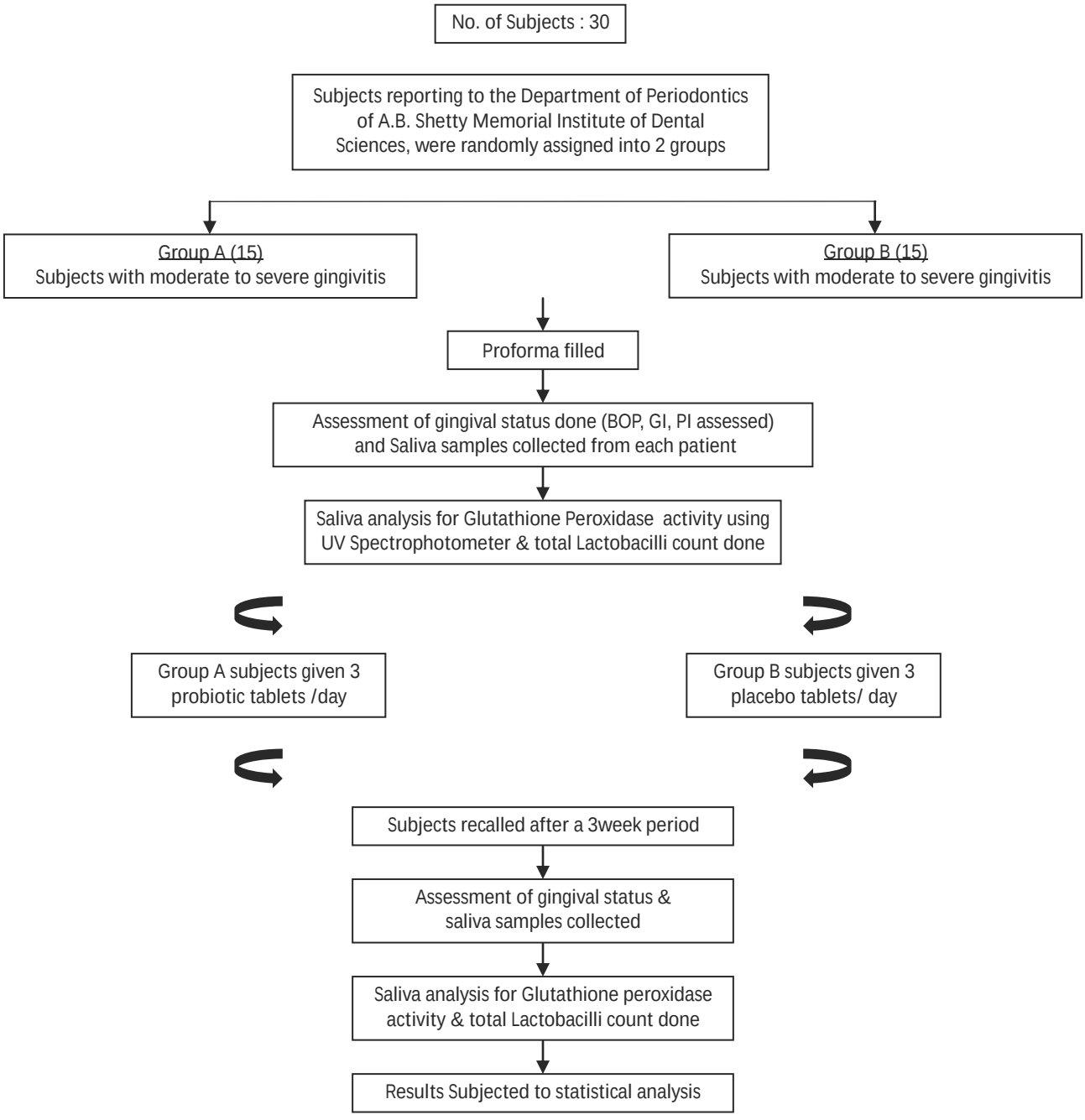
The GPx activity in the probiotic group subjects were significantly lower, ($p<0.0001$) whereas the GPx activity in the placebo group subjects was not statistically different after the experimental period ($p>0.05$).

Discussion

The present double-blind, placebo-controlled, prospective pilot study was designed to study the clinical efficacy and microbial impact of probiotics containing *Bacillus coagulans* (100 CFU/tablet)in the treatment of moderate to severe plaque induced gingivitis. The concept of probiotic bacterio therapy has been proposed for the treatment of periodontal disease due to their ability to inhibit growth of pathogenic bacteria as well as their anti-inflammatory properties. The probiotic tablets in the present study were administered for a 3-week period. [14, 15]

In the present study effects of probiotic bacteria on the periodontal clinical parameters were evaluated. In the probiotic group a statistically significant reduction in GI and BOP was observed (Table II). Our results with respect to GI and BOP agree with previous studies. [16,17] where *Lactobacilli reuteri* was used. In contrast, Staab B et al.,[18] who used *Lactobacillus casei* as a probiotic, reported no statistically significant change. The anti-inflammatory effect of the probiotic may be due to its antagonist action against pathogens by inhibiting the adhesion and growth of

Table I : Experimental Design



pathogens and also exert an influence on local and systemic immune responses. Furthermore, *Bacillus coagulans* have been shown to modulate the immune system by production of short chain fatty acids such as butyric acid and stimulating the production of IL-10.[7,8,15]

In order to determine the inflammatory status of the gingival, glutathione peroxidise activity was evaluated. Glutathione peroxidise is an antioxidant (AO) that catalyses the breakdown of reactive oxygen species (ROS). In health, the balance is maintained among oxidants and antioxidants. Under pathological conditions such as chronic inflammation, a disturbance in favour of ROS production results in oxidative stress. [19]Whole saliva was chosen as the bio fluid to evaluate the levels of Glutathione peroxidise, as it contains gingival crevicular fluid, immune cells, and tissue metabolites and reflects most closely the predominant intra-oral condition.

Table II : Analysis of Gingival Index (GI) scores, Plaque Index (PII) scores, bleeding on probing and Glutathione peroxidise (GPx) activity

Probiotic Group	Placebo Group n=15	P n=15
	Mean ± SD	Mean ± SD
GI baseline GI re-evaluation P -value < 0.0001**	1.6±0.8 1.5±0.1 >0.05 ns*	1.6± 0.2 ns* 1.6±0.3 s **
PI baseline PI re-evaluation P-value >0.05 ns	1.5± 0.6 1.5± 0.2 >0.05 ns	1.4± 0.8 ns * 1.4 ± 0.7 ns
BOP baseline BOP re-evaluation P-value< 0.0001 s**	81.3± 12.6 75.89 ± 11.2 >0.05 ns*	80.5±20.8ns* 79.3± 21.2 s**
Gpx activity baseline (pg/ml) GPx activity re-evaluation (pg/ml) P -value< 0.0001 s**	132.9±21.9 89.7± 15.5 >0.05 ns *	131.0±24.9 131.6±24.6

s**- significant; ns* -non-significant

In this study, salivary glutathione peroxide activity (GPx) indicated a statistically significant decrease (P< .0001) (Table II) following 3 weeks of probiotic therapy in the probiotic group. Similar findings were reported earlier, where it was demonstrated that salivary glutathione peroxide activity increases in subjects diagnosed with plaque induced gingivitis as compared to healthy subjects and is reduced post therapy.[20] The findings observed can be explained as follows; during the initial stages of inflammation, GPx activity is increased to compensate for the increased production of ROS. Following treatment with probiotics, reduction in inflammation was reflected in reduced GPx activity.

In order to determine the influence of probiotics on beneficial oral bacteria, salivary levels of lactobacilli were evaluated. The salivary level of lactobacilli was fairly stable with no statistically significant alterations found between the groups. This indicated that the probiotic has little effect on beneficial bacteria. This result was in agreement with an earlier report,[21] where Lactobacillus reuteri in chewing gum was used.

This study has several limitations such as the following , it was a short term pilot study involving a small patient population, no treatment group was included and only patients with gingivitis were included.

Within the limitations of this study, administration of probiotics containing Bacillus coagulans, demonstrated improvement in the inflammatory status of the gingiva but had minimal effect on Lactobacilli count. In addition, the glutathione peroxidise level was significantly reduced in the probiotic group indicating, a probable beneficial effect of Bacillus coagulans on periodontal health. Further studies involving a larger population and over longer periods are required to confirm these results.

Conclusion

The need to promote health in a natural way has led to increased research on probiotics. Probiotics contain beneficial bacteria which have proven to improve the immunity and absorption of essential vitamins and elements necessary for maintenance of health.

In this pilot study, the probiotic Bacillus coagulans was used in a chewable form in chronic gingivitis patients over a 3-week period during which, there was a significant improvement in the gingival health and noted reduction in glutathione peroxidise activity in the probiotic group. Hence, within the limitations of this study it can be concluded that probiotics can be used as adjuncts to non-surgical periodontal therapy to improve and maintain oral health.

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