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Effectiveness of orally administered probiotic Lactobacillus reuteri in patients with peri-implant mucositis: a prospective clinical study

F. SIGNORINO*[1], N. N. ZOURI[2], G. ALLOCCA[2], C. MAIORANA[3], P. P. POLI[4]

Implant Center for Edentulism and Jawbone Atrophies, Maxillofacial Surgery and Odontostomatology Unit, Fondazione IRCSS Cà Granda Maggiore Policlinico Hospital, University of Milan, Milan, Italy
[1]DDS, MSc
[2]DH
[3]Full Professor and Chairman. Oral Surgery and Implant Center for Edentulism and Jawbone Atrophies, Maxillofacial Surgery and Odontostomatology Unit
[4]DDS, MSc, PhD

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ABSTRACT

Aim The aim of the present prospective clinical study was to evaluate the effectiveness of orally administered probiotic Lactobacillus reuteri in the treatment of peri-implant mucositis.
Materials and methods Eighty patients showing peri-implant mucositis were enrolled and assigned to two different treatment groups. In the test group subjects were instructed to take one probiotic lozenge daily for 30 days. In the control group, patients received placebo. Periodontal indices including plaque index (PI), bleeding on probing (BOP), and probing depth (PD) were clinically recorded around natural teeth and implants at baseline and after a period of 1 month and 3 months.
Results After 3-month evaluation in the test group the differences of plaque and bleeding indices remained statistically significantly lower compared to baseline at both teeth and implants. Contrariwise, no statistically significant differences of PI and BOP were observed in the control group. The intergroup comparison at 3 months yielded statistically significantly lower values for all periodontal parameters around teeth when the probiotic was used. Conversely, no statistically significant differences in periodontal parameters were observed between test and control groups at 3 months around implants.
Conclusions Probiotic intake was effective in reducing PI, BOP, and PD scores around natural teeth and dental implants affected by peri-mucositis, in particular around natural teeth at 3 months.

KEYWORDS Probiotic, Dental Implants, Periodontology

INTRODUCTION

Immediately after teeth eruption, all dental surfaces exposed to the oral environment are colonized by several microorganisms that, interacting with both each other and biologically active proteins and glycoproteins, form a complex biofilm. Dental plaque has different bacterial compositions that may increase the risk of different pathologies affecting not only the periodontium of natural teeth but also peri-implant tissues. Dental implants have been used to replace missing dentition for several years due to high survival and success rates. However, biological complications including peri-implant mucositis are likely to occur, with a prevalence of 19% to 65% of patients (1). Peri-implant mucositis consists in an inflammatory status of the peri-implant mucosa around a functional implant, clinically characterized by bleeding and/or suppuration on probing, and increase in probing depth compared to baseline, without signs of bone loss beyond crestal bone level changes resulting from the initial remodeling (2). The etiology of the inflammatory response lies in the accumulation of bacterial biofilms at the surface of osseointegrated dental implants. This cause-and-effect relationship has been clearly demonstrated in human studies (3, 4). Peri-implant mucositis is considered to be a precursor of peri-implantitis, an irreversible and destructive process that may lead to implant loss (5). For such reason, early identification and treatment of peri-implant mucositis plays a pivotal role in the resolution of the disease (6). Current evidence indicates that mechanical disruption of the biofilm with or without adjunctive use of antiseptic rinses, is usually employed as the treatment of choice (7).
In the search for new alternative treatments to prevent and treat peri-implant disease, the use of probiotic Lactobacillus reuteri has been suggested (8). According

to the World Health Organization, probiotics are defined as living microorganisms which, when administered in a correct amount, confer a health benefit to the host through prevention of adhesion of pathogenic species or inhibition of bacterial growth (9). Indeed, probiotics can inhibit the adherence of pathogenic bacteria in the oral cavity either by forming a barrier via auto-aggregation or by direct co-aggregation with the pathogens (10). These characteristics prompted the use of probiotics to promote oral health by exploiting their ability to decrease the colony forming unit (CFU) counts of oral pathogens (11). Accordingly, probiotics have been used in combination with non-surgical mechanical therapy alone (8, 12, 13) or in combination with antimicrobials (14), antibiotics (15), or photodynamic therapy (16) in the treatment of peri-implant disease.

However, not only the evidence remains limited but also the results are still contradictory with respect to the real efficacy of probiotic agents against peri-implant disease. In view of the aforesaid, the aim of the present prospective study was to evaluate the clinical effectiveness of Lactobacillus reuteri as adjuvant to non-surgical mechanical therapy in the treatment of peri-implant mucositis.

MATERIAL AND METHODS

The study was conducted between September 2016 and June 2018 in the department of Implantology, Fondazione IRCCS Policlinico, University of Milan School of Dentistry (Milan, Italy). It was designed as a monocentric randomized, placebo-controlled, parallel-designed clinical study with a follow-up of 3 months. All procedures were conducted according to the principles outlined by the World Medical Association Declaration of Helsinki as a statement of ethical principles for medical research involving human subjects, as revised, amended, and clarified in its version of 2013. All participants signed a consent form prior to inclusion in the study.

The sample consisted of 80 consecutive healthy non-smoking patients enrolled in a professional oral health maintenance program following implant insertion.

To be included in the study, patients had to present both natural teeth and at least one dental implant loaded with a definitive fixed restoration in function for ≥ 12 months before the recruitment phase, and diagnosed with peri-implant mucositis. The diagnosis of peri-implant mucositis required an inflamed mucosa characterized by bleeding and/or suppuration on probing, and no radiological evidence of radiographic bone loss assessed on orthopantomographs and intraoral radiographs compared to baseline radiological exams performed at the time of the prosthetic loading (2).

Additional inclusion criteria were as follows: patients aged between 18 and 90 years; patients with a non-contributory medical history; patients with no smoking habits (> 10 cigarettes per day); patients who never consumed probiotic agents; patients not presenting peri-implantitis, defined as presence of mucosal inflammation with bleeding and/or suppuration on probing, probing pocket depth ≥ 5 mm and radiographic bone loss of ≥ 2 mm and/or ≥ 3 implant threads. Patients with orthodontic appliances or rehabilitated with implant-retained overdentures were not included in the present study.

At the screening visit (T0), subjects were asked to read and sign a written informed consent, and personal medical history and demographic data were obtained. Each patient underwent periodontal and peri-implant charting with dedicated manual probes (PCP-UNC 15; Hu-Friedy, Chicago, IL, USA and Perio-Probe™, Kerr™, Scafati, Italy for teeth and implants respectively) to register bleeding on probing (BOP), presence of plaque, and probing depths (PD) at four sites per tooth/implant, namely mesial, distal, buccal, and lingual/palatal. In order to detect the plaque, a disclosing agent was used. Data were reported in an electronic periodontal chart (University of Bern periodontal chart, available at http://www.periodontalchart-online.com) to calculate general gingival bleeding index according to Ainamo et al. (17) and plaque control record (PI) according to O'Leary et al. (18). To calculate plaque index and BOP at implant level, a single dichotomous value was assigned to the presence or absence of plaque or bleeding of the implant under study. After data collection, a dental hygienist performed professional prophylaxis to establish a plaque-free dentition. In brief, each patient received professional supra-mucosal plaque removal, sub-mucosal instrumentation using ultrasonic and hand instruments, oral hygiene instructions and motivation.

At this point, patients were randomly assigned to test (n=40) and control (n=40) groups according to a randomized list generated with a computer program (Research Randomizer, Version 4.0; Computer software Retrieved on July 2016, from http://www.randomizer.org/). Following randomization, randomized codes were enclosed in sequentially numbered, identical, opaque, sealed envelopes. The envelopes were opened after the professional prophylaxis by an independent clinician not involved in the selection and treatment of patients. Based on the group allocation, patients in the test group were instructed to assume a tablet of probiotic (BioGaia® ProDentis™, BioGaia®, Stockholm, Sweden) after tooth brushing on a daily basis for 30 days, while patients in the control group received a placebo. Both the probiotic and placebo were presented in identical containers but with different codes according to the randomization. The probiotic was presented in lozenges containing a combination of 2 strains of L. reuteri (DSM-17938 and ATCC PTA 5289) at a dose of 2 x 10^8 CFU/tablet. Subjects in the placebo group took placebo tablets with no active drug substance that had the same appearance, shape, size and taste as the probiotics tablets. In this way, the patient were informed about the nature of the study, but

were blinded with respect to the type of treatment they would receive. At the same time, during the entire study period also the dental hygienist was unaware of the group allocation of the participants according to a double-blind study design.
Patients of both groups were provided with all necessary information in written form. Follow-up appointments were scheduled after 1 month (T1) and 3 months (T2). At each follow-up examination, BOP, PI, and PD values were registered at both tooth and implant level. All data records and treatment procedures were performed by the same dental hygienist blind to the group allocation. During the experimental period, the subjects were encouraged to maintain their normal oral hygiene habits and to continue to brush their teeth twice a day in their own method of brushing, using the same toothpaste to increase reliability of the results.

Statistical analysis

To calculate study sample size, data reported in a previous study were used (19). The authors observed a mean difference in reduction of PD values between probiotic and placebo group of 0.82 ± 0.62 mm. To compute the required sample size, two-tailed Wilcoxon-Mann-Whitney test was performed using statistical software (G*Power 3.1, Heinrich-Heine University, Dusseldorf, Germany). For a test such as the Wilcoxon test, used for follow-up of the clinical parameters over time, with a level of significance of 5% and considering the detection of an effect size of 1.32, a sample size of 11 patients per group affords a statistical power of 0.968 (96%).
Mean scores ± standard deviations (SD) of all clinical parameters were calculated for each subject separately for dental implants and natural teeth. The final data analysis was performed for those subjects who completed the study.
The Shapiro-Wilk test was initially used to assess the normality of data distribution. Because distribution of data did not meet the requirements for normality and homogeneity of variance assumptions, quantitative data were compared between groups using non-parametric statistical tests. The variation of each clinical parameter between T0, T1, and T2 in test and control groups was analyzed with Friedman test separately for natural teeth and implants. In case of statistically significant differences, pairwise comparisons were conducted using Wilcoxon signed rank test to identify any statistically significant changes occurred from baseline within each group. To compare test and control groups separately for natural teeth and implants, the Mann–Whitney U test was used to analyze each clinical parameter at T0, T1, and T2. The significance level was set at $P < 0.05$.

RESULTS

Eighty patients successfully completed the study (48 women, 32 men; mean age 65 ± 12 and 60 ± 15 respectively) (Fig. 1). No patients dropped out of the study because of complications or side effects. Mean values of BOP, PI, and PD are reported in Table 1 and illustrated in Figures 2, 3, and 4.

Intragroup comparison

At T1, a statistically significant reduction of BOP and PI values was observed in all groups. This might be related to the professional oral hygiene procedure performed after data collection at T0 in order to set a plaque-free environment. On the other hand, PD values remained almost unchanged. At T2, different trends could be observed. Comparing the baseline records with the data collected at the end of the study, test group showed a statistically significant reduction of BOP and PI values at both teeth and implants. Conversely, after an initial reduction of BOP and PI registered at T1, the control group showed non statistically significant differences between T0 and T2 at both teeth and implants. Indeed, a statistically significant increase in BOP and PI values between T1 and T2 was observed in the control group. With respect to PD values, non-statistically significant differences were observed during the entire study periods in both groups at tooth and implant levels.

Intergroup comparison

Test and control groups were compared at all study periods for each clinical parameter at both teeth and implant levels. Considering natural teeth, no statistically significant differences in terms of BOP, PI, and PD were observed between test and control groups at T0 and T1. On the other hand, at T2 all clinical parameters resulted in significantly lower values in the test group compared to the control group. This behavior could not be observed when comparing test and control groups at the implant

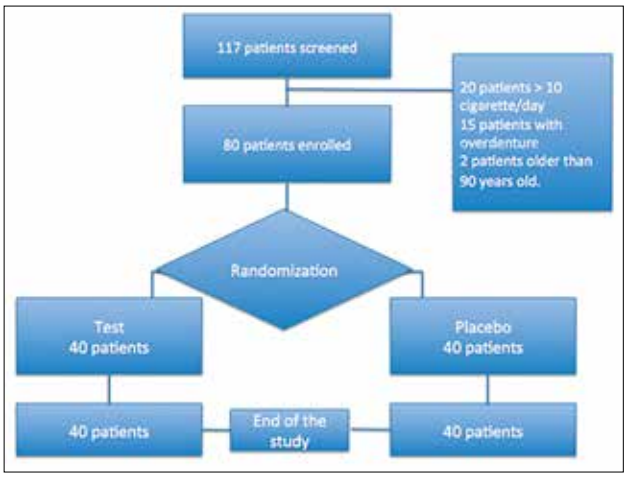

FIG. 1 Flow-chart of the partecipants, 40 patients were randomly assigned to each group. All the patients completed the study.

Plaque index (%)	Baseline (T0)	1 month (T1)	3 months (T2)
Teeth_Test	33,84 ± 20,75	28,05 ± 19,24*	25,76 ± 16,15*^
Teeth_Placebo	38,94 ± 15,72	32,76 ± 14,58*	39,32 ± 18,15°
Implant_Test	56,33 ± 22,96	44,39 ± 20,42*	42,04 ± 19,48*
Implant_Placebo	39,57 ± 22,94	24,37 ± 15,46*	39,88 ± 19,77°
Bleeding index (%)			
Teeth_Test	17,41 ± 12,42	12,02 ± 8,06*	11,92 ± 7,70*^
Teeth_Placebo	20,07 ± 13,85	16,61 ± 11,65*	20,14 ± 11,19°
Implant_Test	40,42 ± 19,69	33,90 ± 15,87*	33,30 ± 16,43*
Implant_Placebo	34,19 ± 22,83	19,16 ± 14,75*	35,18 ± 20,61°
Probing depth (mm)			
Teeth_Test	1,87 ± 0,86	1,87 ± 0,92	1,79 ± 0,61
Teeth_Placebo	1,99 ± 0,67	1,96 ± 0,55	2,03 ± 0,52
Implant_Test	2,22 ± 0,86	2,28 ± 1,14	2,19 ± 0,76
Implant_Placebo	2,30 ± 0,77	2,25 ± 0,59	2,25 ± 0,63

**Statistically significative difference compared to T0.*
° Statistically significative difference compared to T1.
^Statistically significative difference compared to the control group.

Table 1 . PI, BOP, and PD results of all groups with Standard Deviation.

level. While a statistically significant reduction of PI and BOP scores was observed in favor of the test group at T1, no statistically significant differences were observed at T2 for all clinical parameters.

DISCUSSION

The present study was designed to evaluate the clinical effect of probiotic consumption in a cohort of patients rehabilitated with dental implants showing peri-implant mucositis. The objective was to compare BOP, PI, and PD registered at different experimental periods in patients that assumed a probiotic agent versus the same parameters obtained in patients that received a placebo. The probiotic agent was administered in the form of L. reuteri lozenges on a daily basis for 30 days.
The intragroup comparison showed a significant reduction of plaque and bleeding scores from baseline to the first follow-up appointment after 1 month. It is likely that the mechanical therapy contributed substantially in the decrease of PI and BOP values. Nevertheless, a general improvement of plaque and bleeding indices was a common finding in similar studies comparing the

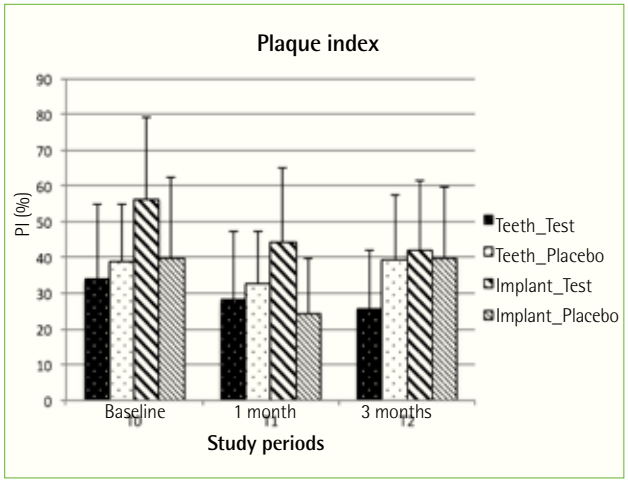

FIG. 2 PI values at baseline, T1 and T2 for dental implants and natural teeth of test and control groups.

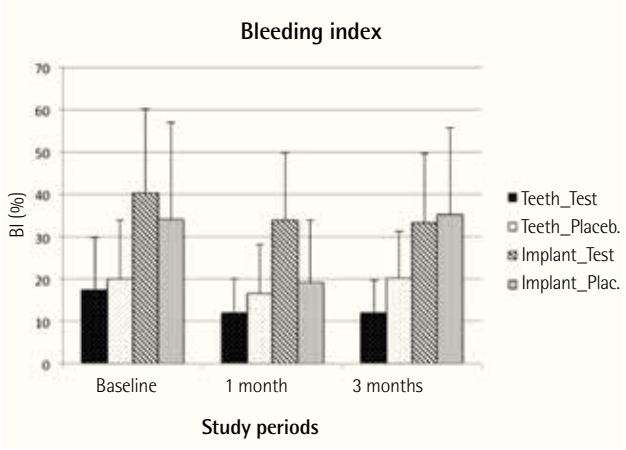

FIG. 3 BOP values at baseline, T1 and T2 for dental implants and natural teeth of test and control groups.

effect of probiotics versus placebo in the treatment of peri-implant disease (8, 12-14). In these terms, the beneficial effect of the probiotic was more masked at the tooth level, where a statistically significant difference between test and control groups could not be observed. On the other hand, patients treated with probiotic showed significantly lower PI and BOP scores compared to the control group at the implant level at 1 month. A similar trend was observed by Galofré et al., who found significant reduction of PI and BOP scores over time following daily assumption of L. reuteri lozenges for 30 days compared to placebo (12). In the said study, however, only BOP scores were statistically significantly lower in the test group compared to the placebo group at 1 month. The rest of the study variables did not differ significantly between groups at 1 month. These results indicate that mechanical disruption of the biofilm may have improved the effect of oral probiotic by promoting the replacement of pathogenic bacteria by beneficial bacteria.

It is worth mentioning however that the adjunctive use of other supplementary treatments to mechanical debridement and probiotic assumption made it difficult to identify the real advantages of probiotics. Mongardini et al. treated implants affected by peri-implant mucositis by means of professionally administered plaque removal, probiotics/placebo, and antimicrobial photodynamic therapy (16). No statistically significant differences in plaque and bleeding scores were observed between probiotic and placebo groups at each observation interval.

Tada et al. treated peri-implantitis lesions with supra-gingival scaling, probiotics/placebo, and azithromycin 500 mg, once a day for 3 days (15). Again, no statistically significant differences in plaque and bleeding indices were found between probiotic and placebo groups at each observation interval. Peña et al. implemented the association of mechanical debridement and probiotics/placebo with 0.12% chlorhexidine mouthwashes (14). According to the previous studies, no significant differences were observed between probiotic and placebo groups at any time point and over time.

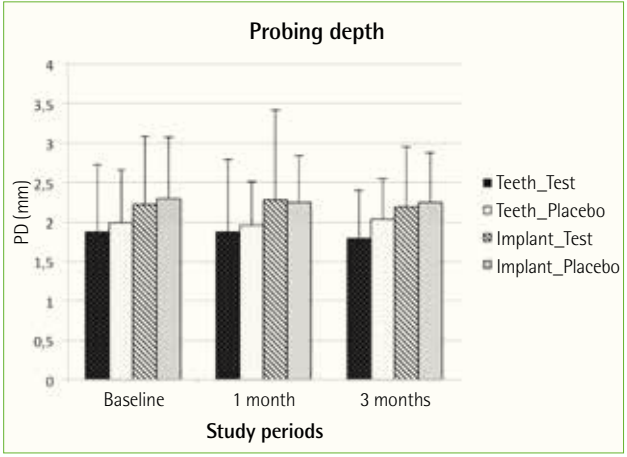

FIG. 4 PD values at baseline, T1 and T2 for dental implants and natural teeth of test and control groups.

In the present study, the intragroup comparison showed that, after probiotic intake, PI and BOP scores remained stable in the probiotic group, with no differences between 1 and 3 months. Contrariwise in the placebo group, PI and BOP scores suffered a slight and progressive increase up to the 3-month follow-up. This trend explained how probiotic consumption resulted in significantly lower plaque and bleeding scores at the end of the observation period compared to baseline. On the other hand, in the placebo group PI and BOP scores almost achieved baseline values, with no statistically significant differences between T0 and T2. Thus, at both teeth and implant levels, the intake of probiotics had positive effects in terms of significant reduction of PI and BOP scores with respect to baseline. This trend has been confirmed by the intergroup comparison of PI and BOP related to natural teeth, where statistically significantly lower values were observed in the probiotic group compared to the placebo group at 3 months. This outcome seems to support the beneficial effect of probiotic on natural teeth. Interestingly, the same intergroup comparison yielded non statistically significant differences between probiotic and placebo at the implant-level.

These findings taken together highlight how dental implants are much more difficult to decontaminate with respect to natural teeth. A reduced effect of probiotic at the implant-level pointed out the difficulty to remove adequately the bacterial biofilm around the implant surfaces. Accordingly, a limited effect of probiotics on intact biofilms has been reported (20). This has been confirmed following microbiological analysis of the bacterial load of Aggregatibacter actinomycetemcomitans, Tannerella forsythia, Porphyromonas gingivalis, Treponema denticola, Prevotella intermedia, Peptostreptococcus micros, Fusobacterium nucleatum, Campylobacter rectus, and Eikenella corrodens in implants with mucositis (12). The only parameter in which a significant decrease was found was the bacterial load of P. gingivalis between baseline and 3 months. The rest of the bacterial loads did not present statistically significant differences between probiotic and placebo. This complies favorably with Hallstrom et al. who did not observe microbiological improvements after mechanical debridement and probiotic intake compared to mechanical therapy alone (13). The limited action of the probiotics on intact biofilms strictly adherent to the implant surface may explain in part the differences observed between teeth and implants in the present study. To overcome such limitation, the study design included, after the baseline recording, a professional mechanical debridement of both natural teeth and dental implants,

in order to establish a plaque free dentition. However, the surfaces of dental implants, often subjected to mechanical and chemical treatments, may represent a perfect environment for microorganisms that become difficult to eradicate. This assumption may explain why adjunctive use of other treatment methods, including antimicrobials, antibiotics, or photodynamic therapy directly targeted against pathogens masked the efficacy of oral probiotics in previously mentioned studies.

It must be noted however that the absence of statistically significant differences from the comparison between probiotic and placebo groups at implant-level could be in part explained by the different average values observed at baseline. Therefore, when interpreting these results, the inverse trends observed from the intragroup comparisons between test and control groups over time may deserve greater relevance.

The question whether the beneficial effect of the probiotic could be maintained for longer periods could not be assessed in this 3-month study. This constitutes a limitation, since the correct time sequence between loss of effect and re-administration could not be assessed. The same issue has been raised by Galofré et al. who observed that the additional improvement of PI and BOP scores obtained immediately after the end of the probiotic treatment, remained constant for up to 90 days (12). In accordance with the present study, it is safe to assume that mechanical debridement allowed probiotics to create a competing biofilm against periodontal pathogens by occupying the space that the latter would tend to occupy. This may explain the clinical stability that could be obtained with the therapeutic use of probiotic agents up to 3 months.

CONCLUSIONS

The results of the present study indicated that probiotic intake was effective in reducing PI, BOP, and PD scores around natural teeth and dental implants affected by peri-mucositis over a time span of up to 3 months. Clear benefit of the probiotic compared to the placebo for all variables could only be verified at 3 months around natural teeth. Conversely at implant level, clinical parameters were not different between probiotic and placebo at 3 months.

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