CLINICAL EFFECTS OF *LACTOBACILLUS REUTERI* PROBIOTIC IN TREATMENT OF CHRONIC PERIODONTITIS. A RANDOMIZED, CONTROLLED TRIAL

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SUMMARY

Aim. The aim of the study is to evaluate the therapeutic efficacy of a probiotic containing *Lactobacillus reuteri* DSM 17938 and *Lactobacillus reuteri* ATCC PTA 5289 in a sample of adult subjects affected by chronic periodontitis, associated with the periodontal treatment of Scaling and Root Planing (SRP).

Methods. The enrolment of 40 patients, aged between 18-70 years (mean age 46.55±14.33), was carried out at Department Diagnosis, Hygiene and Oral Prevention, University of Rome "Tor Vergata", Rome, Italy. The subjects were assessed at baseline (T0) and after 1 month from the SRP (T1). The periodontal evaluation included: last data of Supportive Periodontal Therapy (SPT), Bleeding on Probing (BOP), Pocket Probing Depth (PPD), Clinical Attachment Level (CAL).
The sample of the subjects was randomly divided into two groups: Group 1 (case group), who received the probiotic, and Group 2 (control group).

Statistics. A randomized, single centre, controlled, parallel-group clinical study was conducted. Chi-Square test was used to evaluate statistical differences between gender and Groups. The Anova's one-way analysis and Paired Sample t-test were applied to interpret the changes observed between the groups and in pre and post probiotic assumption for the clinical variables. The significance level was accepted 0.05. All statistical analyses were performed with the SPSS package.

Results. 40 patients aged between 18 and 70 years, with mean age of 46.55±14.33 were analysed. The Groups 1 and 2 were homogeneous, equal distributed for sex (p=0.21) and last data of SPT (p=0.09).
There was a statistically significant difference, between the clinical parameters examined at T0 and T1, for Group 1 and 2: BOP (p=<0.001), PPD (p=<0.001), CAL (p=<0.001). The analysis of the Groups 1 and 2, in relation to the three clinical parameters, at the time T1, shows that for the BOP and PPD there were statistically significant differences (BOP p<0.001), (PPD p=0.02). There was no statistically significant difference between the Groups 1 and 2 in relation to CAL at the time T1 (CAL p=0.11).

Conclusions. The subjects with CP, treated with SRP and probiotic, show some beneficial effect of *Lactobacillus reuteri* with significant reduction of BOP and PPD.

Key words: probiotic, lactobacillus reuteri, chronic periodontitis.

Introduction

The definition of probiotic, now accepted internationally, is elaborated by the International Scientific Association for Probiotics and Prebiotics (ISAPP) which defines probiotics as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host" (1). Probiotics are known to reinforce the immune system, to act on allergies, stress and exposure to toxic substances (2).

Organisms with the following characteristics are considered probiotic (3):

- live micro-organisms, preferably of human origins
- micro-organisms stable and viable after culture, handling and storage before consumption and throughout the expire date
- able to induce a response in the host once inserted into the microbial ecosystem
- micro-organisms safe and not harmful
- able to provide a functional or clinical benefit to the host when consumed.

In dentistry, probiotics have been used to reduction of caries development (4, 5), control of halitosis (6) and periodontal health (7, 8).

The effects of probiotics are both species and strain specific; the most common probiotics belong to two main categories: *Bifidobacterium* and *Lactobacillus* (9).

Bifidobacteria are a group of non-spores, non-motile, catalase-negative, anaerobic, Gram-positive micro-organisms (10). The genus *Lactobacillus* belongs to the phylum *Firmicutes*, class *Bacilli*, order *Lactobacillales*, family *Lactobacillaceae* (11). The *Lactobacillus* species (170 species and 17 subspecies) are anaerobic (optional), catalase-negative, gram-positive, non-spore-forming and often grow better under microaerophilic conditions (12).

The data available indicates that the bacilli of the *Lactobacillus* species are able to modify the composition of the microflora of the oral cavity through antagonistic interactions against potentially pathogenic species, in particular, they can inhibit the growth of some pathogenic periodontal bacteria (13).

The aim of the study is to evaluate the therapeutic efficacy of a probiotic containing *Lactobacillus reuteri* DSM 17938 and *Lactobacillus reuteri* ATCC PTA 5289 in a sample of adult subjects affected by chronic periodontitis, associated with the periodontal treatment of Scaling and Root Planing (SRP).

Materials and methods

A randomized, single centre, controlled, parallel-group clinical study was conducted. The enrolment of 40 patients, aged between 18 and 70 years (mean age 46.55±14.33), was carried out at Department Diagnosis, Hygiene and Oral Prevention, University of Rome "Tor Vergata", Rome, Italy.

The inclusion criteria of the sample were: diagnosis of chronic periodontitis, show for each quadrant at least two elements with PPD ≥4 mm and positive BOP, have at least 20 teeth to include in the assessment, demonstrate willingness to participate for the duration of the study, sign the study consent.

Conversely, the exclusion criteria includes: pregnancy and breast-feeding, antibiotics or other drugs intake that could influence bacterial plaque formation in the last 3 months, physical or mental limits that could limit home oral hygiene, therapies based on particular drugs (immunosuppressants, anti-epileptics, bisphosphonates, anti-inflammatories), previous or present radiotherapy or chemotherapy, missing consent to the study.

The subjects were assessed at baseline (T0) and after 1 month of the treatment (T1), according to the following steps:

1) Screening and selection of subjects

The subjects were selected based on the inclusion/exclusion criteria. A medical history record was compiled and the informed consent was signed, conducted in accordance with the Helsinki Declaration of 1975 as revised in 1983.

2) Baseline (T0) - periodontal evaluation

The periodontal evaluation included the compilation of a periodontal clinical record in which the following parameters were indicated: last data of Supportive Periodontal Therapy (SPT), Bleeding on Probing (BOP), Pocket Probing Depth (PPD), Clinical Attachment Level (CAL).

3) Baseline (T0) - periodontal treatment

SRP was performed using piezoelectric ultra-

sounds and Gracey Standard curettes; the polishing was carried out with a synthetic fibre brush and prophylaxis paste.
All subjects were given instructions for home oral hygiene and motivation. The techniques have been suggested considering the gingival biotype, the presence of gingival recessions and/or enamel abrasions. For the entire duration of the study it was forbidden to perform rinses with antimicrobial solutions and the use of chewing gum.

4) Randomization

The sample of the subjects was randomly divided into two groups: Group 1 (case group) and Group 2 (control group). A person not involved in the clinical trial carried out the randomization. The case group (Group 1) received the probiotic and the control group (Group 2) did not receive the probiotic.
Group 1 was indicated to use the probiotic with two strains of live lactic cultures (*Lactobacillus reuteri* DSM 17938 and *Lactobacillus reuteri* ATCC PTA 5289) according to the specific modalities. The tablets had to be assumed once a day for four weeks and had to be slowly dissolved in the mouth without chewing, preferably away from meals and after accurate oral home hygiene. The pack containing these lactic ferments was delivered immediately after the SRP procedure. The participants received no financial compensation or gifts.

5) Follow-up a 4 weeks (T1) - periodontal evaluation

After 4 weeks from baseline (T0) all subjects returned to perform a periodontal evaluation according to the applied protocol.

Clinical parameters

The clinical variables were examined by single trained investigator. The intra-examiner repeatability was assessed; in fact, the examiner showed 98.7% reproducibility.

Clinical parameters were assessed using a manual probe at six sites per tooth (mesio-buccal, mid-buccal, disto-buccal, disto-lingual, mid-lingual and disto-lingual), excluding third molars.
The clinical variables examined were:

1) *Bleeding on Probing (BOP)*. The BOP was recorded by assigning a binary score (1 for present, 0 for absent) at six sites per tooth and was used to clinically characterize the degree of gingival inflammation. In this registration a bleeding point is considered when bleeding emerges within 10 s after gently probing with a periodontal probe.
   BOP bleeding was calculated as follows: BOP = (number of sites where bleeding is recorded ÷ total number of available surface sites in the mouth) ×100.
2) *Probing Pocket Depth (PPD)*. The PPD were measured in mm as the distance from the gingival margin to the location of the tip of a periodontal probe inserted in the pocket with moderate probing force. The evaluation is carried out at six sites per tooth.
3) *Clinical Attachment Level (CAL)*. The CAL is the distance from the cement-enamel junction (CEJ) to the apical extent of the pocket.

Probiotic product

The patients in Group 1 received tablets with probiotic containing 10^8 CFU of *Lactobacillus reuteri* DSM 17938 and 10^8 CFU of *Lactobacillus reuteri* ATCC PTA 5289, which constitute the active ingredient. Other components present in these tablets are: isomalt (used as a sweetener), hydrogenated palm oil (used as a thickener), flavorings (peppermint, menthol, peppermint essential oil), sucralose (used as a sweetener). One tablet contains no less than 200 million live lactobacilli.

Statistical analysis

Descriptive statistical analysis was conducted to explore the characteristics of the data, calculating the mean and the standard deviation for the clinical quantitative variables. The mean of the variables was compared between the two Groups of patients at each time, and between Time 0 and Time 1 for each Group. Then, to evaluate statistical differences between gender and Groups, the Chi-Square test was used. To interpret the changes observed between the groups and in pre and post probiotic assumption for the clinical variables, the Anova's one-way analysis and Paired Sample t-test were applied. The significance level was accepted 0.05. All statistical analyses were performed with the SPSS package.

Results

40 patients aged between 18 and 70 years, with mean age of 46.55±14.33 were analysed; 50% were females and 50% were males.
The mean age of the participants was 41.3±11.85 years for the Group 1 and 51.8±14.94 years for the Group 2 (p=0.018). Males accounted for 60% of the test group and 40% of the control group.
The demographic and clinical characteristics at baseline are analysed in Table 1.
The Groups 1 and 2 were homogeneous, equal distributed for sex (p=0.21) and last data of SPT (p=0.09).
There was no statistically significant difference between the Group 1 and 2, at baseline examination (T0), in relation to the BOP (87.50±14.75 *vs* 88.45±9.63; p=0.811), PPD (4.12±0.89 *vs* 4.51±0.54; p=0.103) and CAL (4.56±0.94 *vs* 4.95±0.57; p=0.12) (Table 1).
Table 2 highlights the modifications of the clinical parameters (BOP, PPD, CAL) at baseline (T0) and follow-up (T1) in Group 1, Group 2.
There was a statistically significant difference, for Group 1, between the clinical parameters examined at the baseline (T0) and follow-up (T1): BOP (87.50±14.75 *vs* 31.45±15.97; p=<0.001), PPD (4.12±0.89 *vs* 3.47±0.65; p=<0.001) and CAL (4.56±0.94 *vs* 3.94±0.85; p=<0.001) (Table 2).
There was statistically significant difference, for Group 2, between the clinical parameters examined at the baseline (T0) and follow-up (T1): BOP (88.45±9.63 *vs* 58.15±10.38; p=<0.001), PPD (4.51±0.54 *vs* 3.91±0.50; p=<0.001) and CAL (4.95±0.56 *vs* 4.3±0.52; p=<0.001) (Table 2).
The analysis of the Groups 1 and 2, in relation to the three clinical parameters, at the time T1, shows that for the BOP and PPD there were statistically significant differences (BOP 31.45±15.97 *vs* 58.15±10.38; p<0.001), (PPD 3.47±0.65 *vs* 3.91±0.50; p=0.02).

Table 1 - Demographic and clinical characteristics at baseline (T0). Mean values ± standard deviation.

	Subjects tot (n.40)	Group 1 (n.20)	Group 2 (n.20)	*p*-value
Age	46.55±14.33	41.3±11.85	51.8±14.94	0.018* (t-test)
Gender	F:50%, M:50%	F:40%, M:60%	F:60%, M:40%	0.21 (chi-quadro)
Last data of SPT	20.32±18.18	25.2±20.57	15.45±14.33	0.09 (t-test)
BOP (%)	87.98±12.31	87.50±14.75	88.45±9.63	0.811(t-test)
PPD (mm)	4.31±0.75	4.12±0.89	4.51±0.54	0.103 (t-test)
CAL (mm)	4.75±0.79	4.56±0.94	4.95±0.57	0.12 (t-test)

SPT Supportive Periodontal Therapy; BOP Bleeding on Probing; PPD Probing Pocket Depth; CAL Clinical Attachment Level
**Statistically significant (p-value=0.05)*

Table 2 - Clinical parameters at baseline (T0) and follow-up (T1) in Group 1, Group 2. Mean values ± standard deviation.

	Baseline (T0)	Follow-up (T1)	*p*-value
BOP (%)			
Group 1 (n.20)	87.5±14.75	31.45±15.97	<0.001 (Paired Samples t-test)
Group 2 (n.20)	88.45±9.63	58.15±10.38	<0.001 (Paired Samples t-test)
PPD (mm)			
Group 1 (n.20)	4.12±0.89	3.47±0.65	<0.001 (Paired Samples t-test)
Group 2 (n.20)	4.51±0.54	3.91±0.50	<0.001 (Paired Samples t-test)
CAL (mm)			
Group 1 (n.20)	4.56±0.94	3.94±0.85	<0.001 (Paired Samples t-test)
Group 2 (n.20)	4.95±0.56	4.3±0.52	<0.001 (Paired Samples t-test)

BOP Bleeding on Probing; PPD Probing Pocket Depth; CAL Clinical Attachment Level
**Statistically significant (p-value=0.05)*

There was no statistically significant difference between the Groups 1 and 2 in relation to CAL at the time T1 (CAL 3.94±0.85 *vs* 4.3±0.52; p=0.11).

Discussion and conclusion

Periodontal disease is a chronic microbial infection characterized by persistent inflammation, destruction of connective tissue and alveolar bone (14). The presence of pathogenic bacteria, the absence of "beneficial bacteria" and the susceptibility of the host are the main etiological factors of periodontal disease (7). Periodontal destruction is substantially mediated by the host and driven by the bacterial load (15). The pathogenesis of periodontal disease involves a complex interaction between periodontal-pathogenic bacteria and the immune-inflammatory response of the host and is greatly influenced by genetic factors and environmental factors (16). The presence of micro-organisms, even if it is a "conditio sine qua non" for the initiation of the disease, by itself is not a sufficient condition (16). Thanks to technological advances, it has been possible to indicate that, in the aethiology of periodontitis, a microbiological dysbiotic community is at the basis of the onset of chronic inflammation (17). The main management of periodontitis is obtained mainly by removing the causal factors (dental plaque, microbial biofilm, tartar) by SRP and home oral hygiene. Although initially the number of pathogens can be significantly reduced by SRP, the periodontal-pathogenic bacteria are able to quickly re-colonize the treated areas of the oral cavity (15).

Over the years, a number of treatments have been used as adjuncts to SRP to maximize benefits of periodontal therapy (use of antibiotics, antiseptics, probiotic) (18, 19).

The intake of probiotics can offer advantages, if taken as adjuvants in periodontal treatment and determine:

- Direct interactions within dental plaque (colonization resistance). The colonization resistance of pathogenic bacteria could occur for the disruption of plaque biofilm formation (competition for binding sites on host tissues and competition for nutrients). Another mechanism could be the production of antimicrobial compounds, by probiotic species, such as organic acids, hydrogen peroxide, peptides, bacteriocins and anti-adhesion molecules (20). These interactions maintain the oral cavity homeostasis (21), modulate dysbiosis and modify the oral microbiota (22). In a Tekce study, lower values are shown in the proportions of obligate anaerobes from the

analysis by culturing of microbiological sampling performed at baseline and on days 21, 90, 180 in the group who had received probiotic and SRP for CP (23).

- Indirect actions within the oral cavity: modulation of immune function, interaction with immunocompetent cells (macrophages and T-cells), leading to an alteration in the production of cytokines (20). These interactions modulate the inflammatory/immunity destructive response linked to periodontitis (22).

It is reported as a significant decrease in levels of pro-inflammatory cytokines (TNF-α, IL-1β and IL-17) in patients with CP in treatment with probiotic (13).

The biofilms, formed by *Lactobacillus reuteri*, have an immunomodulatory activity to suppress human TNF production by LPS-activated monocytoid cells (24).

According to Ince et al., the low MMP-8 and high TIMP-1 levels may indicate the role of the probiotic in reduction of inflammation-associated markers up to day 180 (25).

The bacterial strains most frequently used as probiotics are lactic bacteria, such as *Lactobacillus reuteri*, which is one of the few endogenous *Lactobacillus* species present in the gastrointestinal tract of vertebrates, Gram-positive and commensal (26).

Lactobacillus reuteri acts in a wide pH range and is resistant to the action of proteolytic and LiPo enzymes (13). The strains of *Lactobacillus reuteri* are acid and bile tolerant and are capable of producing many essential B-complex vitamins, in particular folate (B 9), cobalamin (B 12), but also potentially thiamine (B 1) and riboflavin (B 2) (27).

Many strains of *Lactobacillus reuteri* synthesize the antimicrobial compound reuterin (27). Reuterin (β-hydroxypropionaldehyde) is a potent anti-pathogen compound and is capable of inhibiting a wide spectrum of micro-organisms such as gram-positive bacteria, gram-negative bacteria, fungi, protozoal (24). Moreover, the reuterin is able to block the pathogen's adhesion and prevent its colonization (24).

Quantification of the reuterin was evaluated in order to document the antipathogenic potential of probiotic biofilms; in fact, *Lactobacillus reuteri* biofilms differed in the quantities of reuterin secreted (24).

Several clinical studies (13, 28, 29), reported in their results a significant reduction of periodontal clinical parameters (PI, BOP, PPD) after regular use of probiotics associated with SRP in CP. According to Vivekananda et al. (29), the probiotics can be recommended during non-surgical therapy and the maintenance phase of periodontal treatment as a useful adjunct or alternative to periodontal treatment when SRP might be contraindicated.

According to Teughels et al., the assumption of probiotic supplements for 12 weeks associated with the SRP determines pocket depth reduction ($p<0.05$), attachment gain ($p<0.05$) and Porphyromonas gingivalis reduction (15). This improvement is considered similar to that obtainable following the administration of antibiotics (19).

Although in the systematic review and meta-analysis, the authors state that the probiotics could be a supportive towards managing gingivitis or periodontitis (30, 31), the significant heterogeneity among the studies limits the strength of conclusions regarding the efficacy of probiotics as an adjunct in the treatment of CP (30, 32).

According to a systematic review on the use of probiotics in the treatmentt of periodontal disease, most of the studies show only a short-term benefit with regards to reduction in gingival inflammation and probing depth reduction, but lasting clinical benefits were not seen in any of the studies (18).

In fact, probiotics can ameliorate microbial dysbiosis and produces significant improvement in clinical indicators of disease. However, this effect is often not maintained by the host after the end of probiotic use (22).

A meta-analysis, conducted on randomised controlled trials and patients with CP treated with SRP and probiotic, SRP and placebo or SRP alone, shows some beneficial effect of *Lactoba-*

cillus reuteri with reduction of PPD especially in deep periodontal pockets, CAL gain was similar to other adjuncts (32-34).

Likewise, analyzing the present study and comparing the Groups 1 and 2, in relation to the three clinical parameters (BOP, PPD, LAC), at the time T1, it results that there were statistically significant differences for the BOP and PPD ($p<0.001$, $p=0.02$ respectively), but there was no statistically significant difference in relation to CAL.

This result agrees with the conclusions of the review (32).

The limitations of this study are the small number of enrolled subjects and the duration of treatment limited to four weeks; studies with larger number of patients and longer-term follow-up are needed to confirm these results.

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