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EVALUATION OF THE EFFECTS OF LACTOBACILLI CONTAINING PROBIOTICS IN NON-SURGICAL TREATMENT OF CHRONIC PERIODONTITIS: A CLINICAL AND MICROBIOLOGICAL STUDY

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ABSTRACT

Background & Objectives: There is limited information on the effects of beneficial bacteria (probiotics) on management of periodontal disease. The purpose of the present study was to investigate the clinical and microbiological effects of *Lactobacilli* containing probiotic sachets as an adjunct to non-surgical periodontal therapy.

Methods: Thirty chronic periodontitis patients, who satisfied the inclusion and exclusion criteria, were randomly assigned to: (1) scaling and root planing (SRP) (control group, 15 patients) or (2) SRP plus *Lactobacilli* containing sachets (test group, 15 patients). Full mouth SRP was performed on day 0. On the same day, *Lactobacilli* containing sachets were given to the patients to be taken for 1 month. Periodontal clinical parameters and the proportion of black pigmented anaerobic rods (BPARs) and *Lactobacilli* levels were recorded on day 0, at 1 month and at 6 months post therapy.

Results: Both treatment modalities resulted in a statistically significant improvement in clinical parameters ($p<0.05$) after 1 and 6 months. No intergroup statistically significant differences were observed ($p>0.05$). Microbiological analysis showed a statistically significant reduction of BPARs proportion for both groups, at 1 and 6 months, when compared with baseline values ($p<0.01$). A statistically significant intergroup difference in proportion of BPARs were found at 1 month in favor of the probiotic group ($p<0.05$), however, the intergroup difference was not significant at 6 months evaluation period. A statistically significant negative correlation between clinical parameters (FMBI: $p = 0.032$, PPD: $p = 0.040$) and *Lactobacilli* count was observed. This inverse correlation was also seen with subgingival colonization of BPARs ($p = 0.018$).

Conclusion: Both treatment modalities provided comparable clinical results, however microbiological changes were more evident in the probiotic group. Oral probiotics can repopulate the beneficial microflora and reduce the pathogenic bacteria, however, repeated application is required.

KEYWORDS: Chronic periodontitis, *Lactobacilli*, periodontal treatment, probiotics

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INTRODUCTION

One of the primary objectives of periodontal therapy is reducing the disease-associated pathogens from subgingival biofilm and reestablishing periodontal health and colonization by healthcompatible microorganisms. Numerous treatment modalities have been implicated for periodontal disease, among which mechanical debridement plays the main role, however, some periodontal pathogens may escape treatment due to their ability to invade the periodontal tissues or reside at sites which are inaccessible to the periodontal instruments, so the mechanical therapy may need to be combined with adjunctive modalities. Antibiotics which are delivered either locally or systemically are used as a valuable adjunct to the mechanical therapy [1, 2]. The overuse, misuse and the widespread prophylactic application of antibiotics have led to the emergence of drug resistant micro-organisms. The use of antibiotics may also disturb the indigenous microflora of the body [3].

Time has come to shift the paradigm of the treatment from specific bacteria elimination to modulation of the bacterial ecology through the administration of probiotics [4]. Probiotics are live microorganisms, which, when administered in an adequate amount, confer beneficial effects on human health [5]. The vast majority of probiotic bacteria belong to the genera, *Lactobacilli*, *Bifidobacterium*, *Propionibacterium* and *Streptococcus*. *Lactobacilli* play an important role in the maintenance of health by stimulating the natural immunity as well as by contributing to the balance of the microflora by interacting with the other members of the flora. Studies suggest that *Lactobacilli* as members of resident oral microflora could play an important role in the micro-ecological balance in the oral cavity [6, 7].

Probiotics have been introduced in the field of periodontal healthcare. Krasse et al. [8] reported a decrease in gingival bleeding with the repeated application of the traditional gastrointestinal probiotic *Lactobacillus reuteri*. Staab et al. observed reduction in activity of MMP-3 and elastase enzymes in subjects with plaque-induced gingivitis after consuming probiotic milk containing *Lactobacillus casei* species for a period of 8 weeks [9]. Riccia et al. identified the anti-inflammatory effects of *Lactobacillus brevis* in patients with chronic periodontitis [10]. However, more randomized clinical trials (RCTS) are required to clearly establish the potential of probiotics in preventing and treating periodontal disease. Therefore, the aim of this RCT was to evaluate the effects of oral administration of *Lactobacilli* containing probiotic sachets on clinical and microbiological parameters in patients with chronic periodontitis.

MATERIALS AND METHODS

Subjects and randomization

The study was a masked RCT with a parallel design comparing non-surgical therapy, including scaling and root planing (SRP) (control group) to SRP plus *Lactobacilli* containing probiotic sachets (Lacteol fort, rameda, A.R.E., 10 billions *lactobacillus delbruekii* and *lactobacillus fermentum*) (test group) taken twice daily for one month. The patients for the present study were selected from the outpatients who presented to the Department of Oral Medicine and Periodontology, Faculty of Dentistry, October 6 University, Egypt. The recruitment of patients was carried out from January 2015 to August 2015. The patients were invited to participate and who agreed were explained the selected procedure in detail. A prior written consent was taken from all patients, which was based on the Declaration of Helsinki 1975.

A total of thirty patients of both genders (Twelve males and eighteen females; mean age 36.5 ± 4.42 years) who satisfied the following criteria were included in the study: generalized chronic

periodontitis [11] patients who had sites with a loss of the clinical attachment level (CAL) 3-4 mm, a radiographic evidence of horizontal bone loss and > 20% bleeding on probing (BOP). The patients were excluded if they had debilitating systemic diseases, if they had received antibiotics or anti-inflammatory drugs during the three months prior to the study, if they had received periodontal therapy during the six months prior to the study, if they had dental caries, psychiatric disorders, lactose intolerance and if they were pregnant, lactating or smokers. By block randomization, all patients were allocated immediately before the beginning of the procedures to one of the two treatment groups by the study coordinator who was different from the operator responsible for the clinical procedure. Each group comprised 15 patients. After oral hygiene instructions and full mouth SRP, all patients resumed mechanical tooth cleaning twice daily using a soft tooth brush and the Bass technique. Patients in the test group were instructed to administer the probiotic sachets after brushing.

Clinical parameters

The following clinical measurements were performed on days 0 (baseline; BL), 1 month and 6 months post treatment using Williams graduated periodontal probe at six sites (distal, mid and mesial aspects for both buccal and lingual sites) of each tooth: probing pocket depth (PPD) and clinical attachment loss (CAL) excluding the third molars. Plaque index (PI) [12] and full mouth bleeding index (FMBI) [13] were measured by calculating the percentage of sites that revealed the presence of bleeding on baseline, 1 month and 6 months post treatment. An examiner who was masked with respect to the experimental procedures carried out all measurements of clinical parameters.

Microbiological procedures:

After removal of supragingival dental plaque, the areas corresponding to the deepest pocket of each patient's dentition quadrant were washed with water spray, isolated with cotton rolls, and dried. A sterile paper point # 30 was inserted into the bottom of the periodontal pocket for 30 seconds then placed in sterile Eppendorf tubes containing PBS (phosphate buffer solution, pH 7.4) transport medium, and kept at -4° C until processing. Additionally, unstimulated saliva samples were collected from each patient. Appropriate dilutions were plated onto non-selective 5% horse blood agar plates (Oxoid no. 2; Oxoid Ltd, Basingstoke, England) supplemented with haemin (5 ug/ml) and menadione (0.5 ug/ml) and incubated under anaerobic conditions at 37°C for 5–7 days to determine the count of total cultivable bacteria (TCB) and black pigmented anaerobic rods (BPARs) and on Rogosa agar for 3 days to count lactobacilli [15]. The colonies were identified as *Lactobacillus* based on their growth on Rogosa agar, colonial morphology, Gram staining and by being catalase negative [14]. One examiner collected all microbial samples. The samples were collected on days 0 (baseline; BL), at one month and 6 months post treatment from the same sites. The semi-quantitative colony count was expressed in colony forming units/milliliter (CFU/ml).

Statistical analysis

The null hypothesis was that the adjunctive use of the probiotic sachets to SRP wasn't more effective than SRP in improving clinical or microbiological parameters. All data were recorded as the mean value ± SD for each patient and then for each group at each time point. All parameters were subjected to the Wilcoxon signed rank test to detect intragroup differences and Mann–Whitney U test to detect intergroup differences. The Pearson's correlation coefficient r was calculated to examine the relationship between lactobacilli and BPARs levels. An experimental level of significance was determined at 5 %.

RESULTS

In all, 209 subjects were assessed for eligibility. Among them, 179 individuals did not meet the inclusion criteria. Thirty patients were submitted to initial therapy. The participants were randomly assigned and received the allocated procedure. One patient was lost later during follow-up due to the administration of antibiotic medication for non-dental reasons. The rest of the 29 subjects were included in the statistical analyses. No adverse events had been reported.

The characteristics and baseline clinical parameters of the patients that completed the study (control group, n=14, test group, n=15) are summarized in Table 1. Statistical analysis revealed no differences between the groups at the baseline examination for all the evaluated parameters (p>0.05) (Table 1).

The PI, FMBI, PPD and CAL decreased significantly ($p < 0.05$) in both groups at 1 and 6 months evaluation periods when compared to baseline values. No statistically significant differences were observed between treatment groups at any evaluation period (p>0.05) (Table2).

Microbiological results

The mean percentage of BPARs and salivary and subgingival lactobacilli counts are summarized in table 3. Both treatments led to a significant decrease in the BPARs proportion at 1 and 6 months (Table 3, $p < 0.01$). However, at 1 month evaluation period, the proportion of BPARs in samples isolated from the control group was significantly higher (p< 0.05) than that from the test group. No significant difference between groups was observed after 6 months (p>0.05).

All patients harbored salivary lactobacilli as determined by baseline values. In the test group, there was statistically significant increase in salivary lactobacilli levels when a comparison was performed between baseline and 1 month but not between baseline and 6 months. No statistically significant differences were noted between baseline and 1 and 6 months salivary lactobacilli counts in the control group (p> 0.05) (Table 3). A comparison of the average of colony forming units of salivary lactobacilli between the two groups showed a statistically significant difference in favor of the test group at 1 month but no statistically significant difference had been reported at 6 months.

In subgingival samples, none of the control and test group patients was colonized by lactobacilli at baseline. In the test group, the Lactobacilli counts in the subgingival area increased significantly at 1 and 6 months as compared to the baseline counts. Values reported at 6 month evaluation period were significantly lower as compared to 1 month values ($p < 0.05$).

The presence of lactobacilli in subgingival samples from the test group was inversely associated with values of FMBI and PPD (Table 4, $p < 0.05$). This inverse association was also seen with subgingival colonization of BPARs (Table 4, $p < 0.05$).

TABLE (1) Demographic and baseline clinical parameters of test and control groups

Variable	Control (n=14)	Test (n=15)
Age (years)	35.5 ± 8.43	37.5 ± 7.45
Gender (female)	8	9
PI	1.72±0.34	1.59 ±0.33
FMBI (%)	72.3± 17.73	71.5± 19.7
PPD (mm)	4.55 ± 0.54	4.43 ± 0.62
CAL (mm)	3.81 ± 0.99	3.86 ± 0.81

No significant intergroup differences were observed at baseline (p>0.05). PI plaque index, FMBI full-mouth bleeding index, PPD probing pocket depth, CAL clinical attachment loss.

TABLE (2) Clinical parameters of test and control groups at baseline and follow up periods

Variable		Control	Test	*p*-value (Mann–Whitney U-test)
PI	Baseline	1.72±0.34	1.59 ±0.33	0.16
	1 month	0.86±0.23*	0.78±0.23*	0.696
	6 months	0.83±0.33*	0.79±0.20*	0.733
FMBI (%)	Baseline	72.3± 17.73	71.5± 19.7	0.393
	1 month	16.05 ± 14.44*	11.36 ± 19.53*	0.081
	6 months	22.3± 7.71*	19.7± 8.77*	0.093
PPD (mm)	Baseline	4.55 ± 0.54	4.43 ± 0.62	0.385
	1 month	3.30 ± 03*	3.41 ± 0.5*	0.585
	6 months	3.20 ± 0.5*	2.94 ± 0.43*	0.053
CAL (mm)	Baseline	3.81 ± 0.99	3.86 ± 0.81	0.815
	1 month	2.56 ± 1.07*	3.06 ± 0.87*	0.115
	6 months	2.36 ± 0.37*	2.50 ± 0.97*	0.635

***Significantly different from baseline.** $p \leq 0.05$ **(Wilcoxon signed rank test)**

TABLE (3) Microbiological outcome measures of test and control groups at baseline and follow up periods (values are given as mean +SD)

		Control	Test	*p*-value (Mann–Whitney U-test)
Lactobacilli Saliva ($x10^5$ CFU/ml)	baseline	3.1± 0.6	2.5± 1.9	$p > 0.05$
	1 month	2.7± 1.3	4.7± 1.3*	$p < 0.05$
	6 months	3.2± 1.1	3.3± 0.5	$p > 0.05$
Lactobacilli subgingival ($x10^5$ CFU/ml)	baseline	N.D	N.D	-
	1 month	N.D	0.5 ± 0.7	-
	6 months	N.D	0.2 ± 0.3	-
BPARs (%)	baseline	43.6± 1.0	44.3± 0.4	0.408
	1 month	20.01±4.66*	7.01±7.66*	$p < 0.01$
	6 months	24.37±2.30*	26.11±2.50*	$p > 0.05$

***N.D, not detected. *Significantly different from baseline.** $p \leq 0.05$ **(Wilcoxon signed rank test)**

TABLE (4) Correlations between subgingival lactobacilli level in the test group, and clinical parameters and presence of black pigmented anaerobic rods (Pearson correlation coefficient Rp)

	Correlation coefficient Rp	**P-value**
PI	0.10	0.562
FMBI (%)	- 0.37*	0.032
PPD (mm)	- 0.34*	0.040
CAL (mm)	0.17	0.321
BPARs (%)	- 0.48*	0.018

**** Correlation detected.***

DISCUSSION

The ecological complexity of the oral cavity environment is partly attributed to synergistic or antagonistic interrelationships between different species. In this environment, an equilibrium ratio of beneficial and pathogenic microbes is essential. The recent literature has shown that probiotic administration effectively reduces the number of Streptococcus mutans, suggesting a role for probiotics in caries prophylaxis [(7)]. Additionally, Hatakka et al. [(15)] reported that probiotics also reduced oral Candida counts in the elderly and might offer a new strategy for controlling oral yeast infections. However, only a limited number of studies have examined the effectiveness of probiotics for periodontal diseases. The ability of lactobacilli and other lactic acid bacteria (LAB) to colonize the oral cavity and in particular the periodontium is still debated. This study aimed to identify if oral lactobacilli, with proven probiotic capability in the gastrointestinal tract, are able to improve the periodontal clinical parameters and inhibit the growth of BPARs.

The probiotic tablets tested in the current study (Lacteol fort, rameda, A.R.E., 10 billions *lactobacillus delbruekii* and *lactobacillus fermentum*) were originally manufactured for contributing to the intestinal microbial balance. It is the only probiotic preparation present in Egypt. The selection of *Lactobacillus fermentum* (*L. fermentum*) and *lactobacillus delbruekii* (L. *delbruekii*) was justified by their higher prevelance in the composition of the periodontal microbiota of healthy subjects when compared with that of chronic periodontitis patients [(16, 17)]. Concerning *L. fermentum*, a recent *in vitro* study reported that *L. fermentum* showed stronger inhibitory effects than *Lactobacillus salivarius* and that this species could act as putative probiotic for the periodontium [(18)]. Moreover, a probiotic preparation must contain a specified minimal number of bacterial cells-colony forming units (CFU) per dose. A daily intake of minimum 10^8 – 10^{10} CFU per day is required to show the beneficial health effects [(19, 20)].

Our clinical findings indicated that there was a statistically significant reduction in all clinical parameters in both groups at 1 month evaluation period. The results remained stable over the entire study period (6 months) ($p < 0.05$). The significant reduction of clinical indices observed in the control group proves that the mechanical disruption of the biofilm is the essential step for upsetting the equilibrium and enhancing the replacement of indigenous microbiota.

The results of microbiological analysis demonstrated that the proportions of BPARs decreased in both groups as compared to baseline values. Intergroup analysis demonstrated significantly higher proportions of BPARs in samples isolated from the control group ($p< 0.01$) than that from the test group, however, this effect was reported at 1 month but not at 6 months evaluation period. We concluded that the adjunctive usage of Lactobacilli -containing sachets may lead to better short-term microbiological results. These results are consistent with the results obtained

by Vivekananda et al. [21] and Teughels et al. [22]. Complete eradication of periodontal pathogens following therapy does not commonly occurs, as was observed in the current study, and it is not necessary since successful periodontal treatment should lead to a shift in proportions from a pathogenic to a host compatible periodontal microbiota that should be sustained over time [23, 24].

Few studies assessed the level of Lactobacillus colonization of the subgingival area. In the current study, none of the control and test group subgingival samples was colonized by lactobacilli at baseline. In the test group, the Lactobacilli counts in the subgingival area and saliva increased significantly at 1 and 6 months as compared to the baseline counts. Values reported at 6 months evaluation period were significantly lower as compared to 1 month values however still significantly higher than the baseline values (Table 3). Despite detecting Lactobacilli in subgingival sites and saliva of samples isolated from the patients of the test group at the post therapy evaluation periods, saliva samples had significantly more counts. In the control group, none was colonized by lactobacilli at any evaluation period. This finding is consistent with the results obtained by Koll-Klais et al. [16] who found that only two persons in periodontally healthy group and none in periodontitis group harbored lactobacilli in their subgingival sites indicating that the subgingival region is not a common habitat for lactobacilli.

Comparison with other studies is difficult since studies using probiotics in patients with chronic periodontitis present a high degree of heterogeneity in the probiotic strains, dosages, vehicles of administration, modes of administration and duration. The results obtained in this study indicate that the studied probiotic was able to substantially affect the levels of BPARs, however, the oral route of administration didn't provide sustained contact with oral tissues. A lozenge form or chewing tablet or gum might facilitate probiotic adhesion to dental tissues to become a part of the biofilm and better serve the needs for periodontal health prophylaxis [7]. Controlled clinical trials and long term studies are required to investigate the best form and concentration of probiotics bacteria. In biofilms, probiotics may have to be continuously administered for prolonged effects .However; further studies are needed to determine if this is applicable. Moreover, despite the favorable microbiological results, it would be difficult to definitely attribute increased lactobacilli levels to the administered probiotic since more microbiological analysis up to the strain level should be done.

The current results could be useful to produce an application able to eradicate the putative periodontal pathogens in the periodontium. The possibility to manage periodontal disease with a natural, non-invasive method is particularly appealing, and may prevent problems related to pharmacological treatments, such as the use of antibiotics.

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