



Evaluation of the Effect of Topically Applied Probiotics on Interleukin-10 and Osteoprotegerin as an Adjunctive Treatment of Stage I and II Grade A Periodontitis Cases

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KEYWORDS

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level (CAL).

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ABSTRACT

Aim: the use of probiotic gel may be considered as beneficial treatment for periodontitis. This study was directed to evaluate the possible effect of topically applied probiotics on Interleukin-10 (IL-10) and osteoprotegerin (OPG) combined with scaling and root planning (SRP) in treatment of stage I and II grade A periodontitis. **Subjects and Methods:** 18 female patients were divided randomly into two equal groups. Group I (PG): received SRP combined with topically applied probiotics gel. Group II(CG): received SRP only. All patients were received phase I periodontal therapy. Then all patients were evaluated clinically and biochemically at base line, one month and three months after periodontal therapy Results recorded, tabulated and statistically analyzed using SPSS (statistical package for social sciences). **Results:** Clinical parameters such as plaque index (PI), gingival index (GI), probing pocket depth (PPD) and clinical attachment level (CAL) were evaluated. In all groups there was a significant reduction regarding the clinical parameters. There was insignificant better results regarding PI and GI in group I. while, there was significant better results regarding PPD and CAL in group I than group II. OPG levels showed insignificant better results in PG than CG but IL-10 levels showed significant better results in PG over time than CG. **Conclusion:** Adjunctive topically applied probiotic gel exhibited anti inflammatory effect through the significant expression of IL-10 after treatment of stage I and II grade A periodontitis patients. On the other hand seemed to be non significantly improve the bone formation through the limited expression of OPG

INTRODUCTION

Periodontitis is an inflammatory disease of the periodontium which is characterized by a progressive destruction of the tissues supporting the tooth. The disease is currently considered to progress as periodic, relatively short episodes of rapid tissue destruction followed by some repair, and prolonged intervening periods of disease remission. Despite the apparent random distribution of episodes of disease activity, the resulting tissue breakdown exhibits a symmetrical pattern of alveolar

bone loss and pocket formation which is common to several forms of periodontitis⁽¹⁾. It has become apparent that the pathogenesis of periodontal diseases is more complex than the presence of virulent microorganisms. To date, the bulk of evidence points to the host response to bacterial challenge as a major determinant of susceptibility⁽²⁾. While pro-inflammatory cytokines include interleukin -1 α (IL-1 α), IL-1 β , IL-6, IL-7, tumor necrosis factor- α (TNF- α) contribute to acute and chronic periodontal inflammation and tissue injury, the second group with antagonist effects is formed by cytokines such as IL-10, IL-4, interleukin-1 receptor antagonist (IL-ra) called anti-inflammatory cytokines⁽³⁾.

Interleukin-10, has a central role in infection by limiting the immune response to pathogens and thereby preventing damage to the host⁽⁴⁾ and inhibits the ability of macrophage but not B cell antigen presenting cells (APC) to stimulate cytokine synthesis by T helper1 (Th1) T cell clones. Furthermore, IL-10 appears to be a more potent inhibitor of monokine synthesis than IL-4 when added at similar concentrations⁽⁵⁾. Osteoprotegerin (OPG), a secreted member of the tumor necrosis factor receptor superfamily, has a variety of biological functions which include the regulation of bone turnover. OPG is a potent inhibitor of osteoclastic bone resorption and has been investigated as a potential therapeutic for the treatment of both osteoporosis and tumor-induced bone disease⁽⁶⁾.

Scaling and root planing (SRP) still and remain the gold standard for treatment of periodontitis. Nevertheless, different therapeutic strategies have been proposed to improve the results of SRP and hence to avoid the need of periodontal surgical interventions in some patients with advanced periodontitis⁽⁷⁾. Probiotics are live nonpathogenic microorganisms administered to improve microbial balance⁽⁸⁾. Probiotic bacteria have strain-specific anti-inflammatory effects in healthy adults⁽⁹⁾. Recently, probiotics are suggested to have the ability to reduce inflammatory factors and to maintain bones in various ways⁽¹⁰⁾.

METHODOLOGY

This study was designed as a randomized, controlled clinical trial carried out on 18 female patients ranged in age from 24-57 years who was diagnosed clinically and radiographically as Stage I or II Grade A Periodontitis. All Patients in this study was selected from those attending at the outpatient clinic, Oral Medicine, Periodontology, Oral Diagnosis and Dental Radiology Department Al-Azhar University, Assiut branch.

All patients in this study

1. Had stage I or II Grade A periodontitis with probing pocket depth \leq 5mm.
2. Free from any systemic diseases⁽¹¹⁾.
3. Didn't receive periodontal therapy, systemic antimicrobials or anti-inflammatory drugs in the last 3 months prior the beginning of the study.
4. Didn't received systemic drugs that modifying the bone remodeling at least 12 months prior to beginning of the study.

The selected patients were divided randomly by flipping a coin into two equal groups: -

Group I (probiotic group): patients received conventional periodontal therapy (scaling and root planing) combined with topically applied probiotics gel.

Group II (control group): patients received only conventional periodontal therapy (scaling and root planing).

Clinical evaluation:

Patients were clinically evaluated at baseline before SRP and before probiotic application then after 1 and 3 months by the following parameters: - Gingival Index (GI), Plaque Index (PI), Probing Pocket Depth (PPD), Clinical Attachment level (CAL).



Biochemical evaluation:

Gingival crevicular fluid (GCF) samples were collected at baseline, after 1 and 3 months for assessment of IL-10 level and OPG level using enzyme-linked immune-sorbent assay (ELISA).

Sample collection and storage:

- Selected sites and the adjacent teeth were isolated with cotton rolls to prevent the contamination of the samples with saliva. Paper point ISO #30 taper 0.02mm/mm was inserted slowly with a sterile tweezer into the pocket.
- The paper point was left for 30 secs, then it was carefully removed without touching any adjacent unrelated tissues then transferred to a sterile Eppendorf tube containing 1 ml of a phosphate buffer solution (PBS).
- Samples stored frozen at -80°C until further biochemical analysis.

Probiotic preparation:

- ProlacSan® Gel syringe contains probiotic powder and thickener sealed in a metal foil. Each syringe contains a total of 6×10^9 (CFU) of *Lactobacillus brevis* and *plantarum*.
- For preparation, aspirate distilled water maximum 1.2ml, shake and wait minimum 5 minutes and ideally for 15 minutes.

Periodontal intervention:

All patients were received phase I periodontal therapy including scaling and root planing and oral hygiene instructions.

Probiotic application for the test group:

Following conventional periodontal therapy (SRP):

- Isolation with cotton rolls for the selected site and thoroughly dryness before gel application.

- The gel was applied carefully subgingivally into the pocket until excess gel was observed from the gingival margin and excess gel was removed (fig.1).



Fig. (1) Showing probiotic gel application

Post treatment instructions:

Patients who received probiotics were instructed not to rinse, eat or drink for at least 2 hours, not to disturb the area with tongue, finger or toothpick, not to chew any hard, or sticky food for at least 1 week, postpone brushing and flossing on the treated site for few days.

Statistical analysis

The data were collected, tabulated, computed and statically analyzed.

RESULTS

The effect of different treatment modalities on the clinical and the biochemical parameters were illustrated in table (1).

Changes in Gingival index and Plaque index :

- There was significant steady decrease in the GI and PI over time from baseline to 3 months in both groups.
- There were no statistically significant differences between the two groups at baseline, 1 month and 3 months.

Table (1) The mean \pm standard deviation (SD) and p-values of GI, PI PPD, CAL, OPG, IL-10 levels at different intervals:

Clinical and biochemical parameters		Group I		Group II		P-value*
		Mean	SD	Mean	SD	
GI	Base line	2.49	0.3	2.41	0.4	=0.637
	1 month	0.89	0.4	1.02	0.5	=0.553
	3 months	0.98	0.9	1.03	0.6	=0.902
	P-value*	< 0.001		< 0.001		P=0.350
PI	Base line	2.64	0.5	2.58	0.6	=0.802
	1 month	0.53	0.1	0.70	0.1	=0.352
	3 months	0.93	0.2	1.11	0.6	=0.599
	P-value*	< 0.001		< 0.001		P=0.404
PPD	Base line	4.09	0.9	3.90	0.9	=0.663
	1 month	2.64	0.5	2.66	0.5	=0.966
	3 months	2.13	0.6	2.53	0.4	=0.040
	P-value*	< 0.001		=0.002		P=0.046
CAL	Base line	2.67	0.7	2.28	0.8	=0.304
	1 month	1.26	0.9	1.43	0.8	=0.652
	3 months	0.73	0.1	1.38	0.7	=0.026
	P-value*	< 0.001		=0.011		P=0.010
OPG	Base line	4.54	0.8	4.41	0.5	=0.675
	1 month	4.44	0.6	4.57	0.7	=0.704
	3 months	5.31	0.7	4.93	0.7	=0.239
	P-value*	=0.166		=0.268		P=0.616
IL-10	Base line	93.04	8.3	93.76	9.1	=0.945
	1 month	88.64	8.6	103.67	13.3	=0.132
	3 months	130.7	14.5	106.2	11.9	=0.029
	P-value*	=0.002		=0.437		P=0.028

Changes in Probing Pocket depth (PPD) and clinical attachment level (CAL) :

- There was significant steady decrease in the PPD and CAL over time from baseline to 3 months in both groups.
- There were no statistically significant differences between the two groups at baseline and 1 month. There was statistically significant difference between the two groups at 3 months. For the interaction between time and treatment group, there was significant difference better results in group I over time than group II.

Changes in OPG level:

- Group I: There was insignificant increase in the OPG level over time from baseline to 3 months

where $p=0.166$. A statistically insignificant difference between base line and 3 months ($p=0.161$). While baseline was insignificantly higher than 1month ($p=0.699$).

- Group II: The level of OPG had insignificant raise over the study period
- There were no statistically significant differences between the two groups at baseline, 1 month and 3 months

Changes in IL-10 Level:

- Group I: There was significant increase in the IL-10 level over time from baseline to 3 months where $p=0.002$. A statistically significant difference was found between baseline and 3months and between 1month and 3months.



On the other hand, baseline was insignificantly higher than 1 month ($p=0.745$).

- Group II: The IL-10 level presented insignificant increase over the study period (baseline (93.8 ± 9.1), at 1 month (103.7 ± 13.3) and at 3 months (106.2 ± 11.9) where $p=0.374$).
- There were non-statistically significant differences between the two groups at baseline, 1 month. While the IL-10 was significantly higher at 3 months in group I than group II ($p=0.029$). For the interaction between time and treatment group, there was significant ($p = 0.028$) better results regarding IL-10 level in group I over time compared with group II.

DISCUSSION

The key features of periodontitis immune disruption include excessive inflammation that fails to resolve becoming chronic and self-destructive in nature, generating an environment that is favorable to pathogenic bacteria⁽¹²⁾. Cytokines central regulatory role is evident in the inflammatory process and immune cell response that underlies bone destruction in periodontitis⁽¹³⁾. Anti-inflammatory cytokines play an important role in the pathophysiology of periodontitis, while the initiation and progression of periodontal inflammation could be due to deficient or inconvenient response of the anti-inflammatory cytokines⁽¹⁴⁾.

IL-10 is a very important multi potent anti-inflammatory cytokine that showed many positive effects controlling pro-inflammatory cytokines such as IL-1 β , TNF- α , and IL-6 secretion, reducing the inflammatory destructive effects such as bone resorption in addition to enhancing periodontal healing process⁽¹⁵⁾. OPG is another key factor in periodontal disease pathogenesis, acts as a decoy RANKL receptor, which suppresses osteoclast differentiation. The RANKL-OPG system is a crucial regulator of osteoclastogenesis and bone resorption in both physiological and pathological

conditions⁽¹⁶⁾. The reduced RANKL/OPG ratio in sites with destructive periodontal activity after periodontal treatment supports using of these molecules in periodontal diagnosis, monitoring and treatment⁽¹⁷⁾.

In 2017 classification, periodontitis diagnosis using the pluri-dimensional classification according to stages and grades leads to a more personalized patient-centered treatment that also takes into consideration the patient's medical history^(18,19). Stage I and II periodontitis were selected in this study. It has an excellent prognosis if intercepted early but can cause severe damage to the dentition with delay in therapy⁽¹⁸⁾. Non-surgical periodontal therapy remains the corner stone of periodontal treatment aiming to remove the etiologic factor, thereby stopping the disease progression and re-establishment of biologically acceptable root surface for healing. So it is not only the first mode of periodontal disease treatment but it also restores tissue health^(19,20).

Probiotics was locally delivered as an adjunctive treatment to the basic periodontal therapy. It could be used as a monotherapy or as an adjunctive treatment not only to prevent infections but also to play a role in disrupting the microbial pathways that result in inflammatory immune disorders. The ease of administration of probiotics and the fact that no adverse outcomes have been reported in the literature has promoted increasing interest from the research community for this preventive approach in a number of diseases, including periodontal diseases⁽²¹⁾. Probiotics have the ability to improve alveolar bone and attachment level, microbiological, and immunological outcomes in the treatment of periodontitis in animal models⁽²²⁻²⁴⁾.

In this study a gel form of probiotics containing *Lactobacillus brevis* and *plantarum* that was delivered by a syringe to introduce the gel to the depth of the pocket as close as possible providing a more effective approach. Specific strains of probiotic bacteria including *L. plantarum* GOS42 have

potential for use in oral care products to reduce gingival inflammation, they showed downregulation of inflammatory mediators released by *ex-vivo* human monocytes in response to bacterial LPS, with potent anti-inflammatory effects, which were largely independent of their viability⁽²⁵⁾. A more recent study in 2022 presented data of a locally applied muco-adhesive gel enriched with *Lactobacillus* probiotic strains to prevent and treat periodontitis⁽²⁶⁾.

The present study excluded smokers, pregnant, lactating and medically compromised patient because they are considered as very effective risk factors in periodontal disease. Furthermore, Patients received periodontal therapy, taking antimicrobials or anti-inflammatory drugs in the last 3 months and patients received bone remodeling drugs for the 12 months prior the study were also excluded as it is more probably will provide unreliable or inaccurate results in this research according to guide lines in periodontal therapy⁽²⁷⁾.

In the present study clinical and biochemical evaluation were used in the assessment of inflammatory and bone turnover biomarkers through collecting and analyzing gingival crevicular fluid that can be considered as an adjunct to the traditional methods of periodontal diagnosis as they are vital in identifying the subjects at risk for future periodontal breakdown in addition to determining the therapeutic outcomes⁽²⁸⁻³⁰⁾.

Regarding plaque and gingival index, statistically significant decrease over the study period from baseline to 3 months in both groups. There were non-statistically significant differences between the two groups at baseline but there were better results in the probiotics group without statistical significant when compared with control group. These results corresponds with findings of different studies showed significant improvement in PI and GI after probiotic supplementation^(28,31,32). Another study indicated that a daily consumption of a probiotic milk reduces the effects of plaque-induced gingival inflammation in patients with a higher plaque

score^(33,34-37). While another recent study suggested that the use of probiotics seemed not to be beneficial for prevention of plaque accumulation as it could be affected by reduced oral hygiene⁽³⁸⁾. The use of probiotic tablets containing *L. plantarum*, *L. brevis* and *P. acidilactici* did not lead to significant changes in mean GI otherwise, a significant reduction occurred in the number of sites with higher GI and severely inflamed in addition to a significant microbiological impact have been promoted only in the test group⁽³⁹⁾. These clinical results could be attributed to mechanical debridement, patient motivation and education about oral hygiene measurements during observation period that was provided to both groups⁽⁴⁰⁾.

Pocket depth and clinical attachment loss measurements in the present study were significantly steady decreased over time from baseline to 3 months in both groups. While there were no statistically significant differences between the two groups at baseline and 1 month, a statistically significant difference was found between the two groups at 3 months as well as better results regarding PPD and CAL in probiotic group I over time compared with control group. These results consistent with many studies showed significant clinical improvement associated with PPD reduction as well as CAL gain^(31,32,36-38). Furthermore, improvement in gingival crevicular fluid volume, and reduction in the periodontopathogen load and pro-inflammatory markers in periodontally diseased patients⁽³⁷⁾. Another study stated that there was significantly more pocket depth reduction and attachment gain ($p < 0.05$) in moderate and deep pockets⁽⁴¹⁾. While using dietary supplement in a form of suspension containing *L. salivarius* SGL03 suggested that, it could be able to reduce pocket depth despite the lack of changes in other clinical parameters and the number of bacteria in supragingival plaque⁽⁴²⁾. In addition, the oral administration of *L. reuteri* Prodentis improved the short-term clinical outcomes included bleeding on probing and pocket probing depths in non-smoking patients



with initial-to-moderate chronic periodontitis⁽³²⁾. Another study used probiotics showed significantly more pockets converting from ≥ 4 mm at baseline to ≤ 3 mm at 24 weeks and less sites in need for surgery ($4 \pm 4\%$ versus $8 \pm 6\%$)⁽⁴³⁾.

Regarding the biochemical evaluation probiotic group provided significant increase in the IL-10 level over time from baseline to 3 months with a statistically significant difference between baseline and 3 months. On the other hand, control group presented insignificant increase over the study period. There were non-statistically significant differences between the two groups at baseline regarding IL-10 level but a significant better results regarding IL-10 level in PG over time compared with CG specially after 3 months ($p=0.029$). These results in agreement with a study used supplementation of probiotics *L. rhamnosus* and *L. acidophilus* and exhibited a significant effect on the severity of apical periodontitis in rats, demonstrating the anti-inflammatory effect of probiotics on the development of apical periodontitis as IL-10 was significantly more immunolabeled in the probiotic groups^(44,45). A study in 2022 used probiotic complex stated that the IL-10 level was significantly increased in the probiotic group⁽⁴⁶⁾.

There is a great conflict among many studies revealing many variations regarding the OPG level. As well as confirming the probiotic effect on RANKL/RANK/OPG pathway requires extensive studies, including animal and human studies⁽²¹⁾. While the expression level of OPG after non-surgical periodontal treatment showed no significant changes compared with healthy subjects⁽⁴⁷⁾. Furthermore, another study suggested that probiotics could reduce inflammatory factors (for example TNF- α and IL-1 β) and increase bone OPG expression⁽⁴⁸⁾. An investigation demonstrated that treatment with *Lactobacillus para* or the *Lactobacillus mix* modifies immune response in bone by decreasing cytokine and enhancing OPG expression⁽⁴⁹⁾. In a study on periodontal disease, the probiotic supplementation did not decrease the RANKL expression while OPG

significantly increased⁽⁵⁰⁾. However, in another one there was a decrease in RANKL level while OPG level remained stable⁽⁴⁶⁾.

Among all these studies this present study observed that OPG level started with a non-statistically significant differences between the two groups at baseline, PG and CG showed steady non-significant raise over the study period with better results regarding PG. This Conflict in results may be due to the difference in species used as the enhanced OPG levels came usually with certain lactobacillus species more than others and as well as the percent provided from each species.

CONCLUSIONS

- Adjunctive topically applied probiotic gel exhibited anti inflammatory effect through the significant expression of IL-10 after treatment of stage I and II grade A periodontitis patients.
- Probiotic gel application seemed to be non significantly improve the bone formation through the limited expression of OPG after periodontal therapy.
- The adjunctive use of probiotic gel resulted in significant reduction of probing pocket depth and gain in attachment level in non-surgical periodontal treatment of stage I and II grade A periodontitis.

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تقييم تأثير البروبيوتك موضوعيا على انتروكوكين 10 و استوروجين كعلاج مساعد في المرحلة 1 و 2 التصنيف ا لحالات التهاب اربطة الاسنان

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الملخص:

الهدف: يمكن اعتبار استخدام هلام البروبيوتك علاجًا مفيدًا للتهاب دواعم السن. تم توجيه هذه الدراسة لتقييم التأثير المحتمل للبروبيوتك المطبق موضعياً على انتروكوكين 10 و استوروجين جنباً إلى جنب مع كحت وتخطيط الجذر في علاج المرحلة و من التهاب اللثة

المواد والاساليب: تم تقسيم 18 مريضة بشكل عشوائي إلى مجموعتين متساويتين. المجموعة الأولى (PG): تلقى SRP مع جل البروبيوتك المطبق موضعياً. المجموعة الثانية (CG): استلمت SRP فقط. تم استقبال جميع المرضى من المرحلة الأولى من علاج اللثة. ثم تم تقييم جميع المرضى سريريًا و كيميائيًا حيويًا عند خط الأساس . بعد شهر واحد وثلاثة أشهر من تسجيل نتائج علاج اللثة وجدولتها وتحليلها إحصائيًا باستخدام SPSS (الحزمة الإحصائية للعلوم الاجتماعية).

النتائج: تم تقييم العلامات السريرية مثل مؤشر البلاك (PI) ، مؤشر اللثة (GI) ، فحص عمق الجيب (PPD) ومستوى التعلق السريري (CAL). في جميع المجموعات كان هناك انخفاض كبير فيما يتعلق بالمعايير السريرية. كانت هناك نتائج غير ذات دلالة أفضل فيما يتعلق ب PI و GI في المجموعة الأولى . بينما كانت هناك نتائج أفضل مهمة فيما يتعلق ب PPD و CAL في المجموعة الأولى من المجموعة الثانية. أظهرت مستويات OPG نتائج أفضل في PG مقارنة ب CG ولكن مستويات IL-10 أظهرت نتائج أفضل في PG مرور الوقت من CG.

الخلاصة: ظهر جل البروبيوتك المساعد موضعياً تأثيراً مضافاً للتهابات من خلال التعبير الهام لـ IL-10 بعد علاج مرضى التهاب دواعم السن من الدرجة الأولى والثانية. من ناحية أخرى . يبدو أنه لم يحسن بشكل كبير تكوين العظام من خلال التعبير المحدود لـ OPG

الكلمات المفتاحية: البروبيوتك ، إنتروكوكين 10 - (IL-10) ، أوستوبروتيجرين (OPG) ، التهاب اللثة . مستوى التعلق السريري (CAL).

