Evolution of Salivary Osteoprotegerin OPG and RANKLE after using Probiotics in Diabetic Patients Type 2 with Periodontitis

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Abstract

Aim: Probiotics may be a useful treatment for periodontitis. The purpose of this study was to determine the potential effect of topically applied probiotics on RANKLE and osteoprotegerin (OPG) combined with scaling and root planning (SRP) in the treatment of stage I, II, and III grade periodontitis in type 2 diabetic patients. **Subjects and Methods:** 50 patients (28 females and 22 males) were randomly allocated into three groups. Group I has simple gingivitis. Group II includes those with mild gingivitis. Group III: with severe gingivitis. Another 50 patients (30 female and 20 male) were randomly allocated into three groups. Group I has simple periodontitis. Group II includes those with mild periodontitis. Group III: with severe periodontitis. In addition, 25 people were collected as a control group (15 women, 10 men). All gingivitis patients in the group were given systemic probiotics in tablet form. While periodontitis patients received a topically administered probiotic gel. And complete phase I periodontal therapy. All patients were examined clinically and biochemically at baseline, one month, and three months following periodontal therapy. The results were collected, tabulated, and statistically analysed using SPSS. **Results:** Clinical indicators such as plaque index (PI), BP, PPD, and CAL were assessed. In all groups, there was a significant drop in the clinical measure OPG and raised RANKLE. Group I had significantly better results in terms of PPD and CAL compared to group II; however, there was no significant difference in PI. OPG levels showed insignificantly better outcomes in PG, but RANKLE levels showed significantly better results in CAL with time than PI. **Conclusion:** Adjunctive topically administered probiotic gel had an anti-inflammatory impact by significantly increasing RANKLE expression in patients with stage II and III grade A periodontitis. In contrast, reduced OPG expression appeared to considerably promote bone growth.

Keywords: Probiotics, Osteoprotegerin (OPG), RANKLE (RANKL), Type 2 Diabetes, Periodontitis.

Introduction

Periodontitis is an inflammatory condition that affects the periodontium, leading to a gradual deterioration of the tissues that support the tooth. The disease is currently seen as exhibiting periodic progression.

Episodes of fast tissue damage, followed by partial healing, and long periods of illness remission. Although the bouts of disease activity may seem random, the ensuing tissue breakdown shows a symmetrical pattern of alveolar bone loss and pocket development. This pattern is commonly seen in many types of periodontitis.[1] Evidence suggests that the development of periodontal disorders involves a more intricate process than simply the existence of harmful germs. Currently, the majority of research indicates that the way the host responds to a bacterial challenge is a

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significant factor in determining their sensitivity to it.[2] RANKLE test, also referred to as tumor necrosis factor ligand superfamily member (TNFSF11) or TNF-related activation-induced cytokine (TRANCE),[3] plays a crucial role in infection by restricting the immune response to pathogens, thus protecting the host from damage. It specifically hinders the ability of macrophage antigen-presenting cells (APC) to induce cytokine production in T helper 1 (Th1) T cell clones, while not affecting B cell APCs. Osteoprotegerin (OPG) is a secreted protein that belongs to the tumour necrosis factor receptor superfamily.[4,5] It plays a crucial role in regulating bone turnover and has various other biological roles.[6] OPG is a powerful substance that effectively stops the breakdown of bone by osteoclasts. It has been studied as a potential treatment for both osteoporosis and bone diseases caused by tumors. Scaling and root planing (SRP) continue to be the most effective and widely accepted treatment for periodontitis. However, many therapeutic approaches have been suggested to enhance the outcomes of scaling and root planing (SRP) and, as a result, prevent the necessity of periodontal surgical procedures in certain patients with advanced periodontitis.[7] Probiotics are living microorganisms that are not harmful and are given to enhance the balance of microorganisms.[8] Probiotic bacteria exhibit strain-specific properties that can reduce inflammation in healthy adults.[9] Probiotics have recently been proposed to possess the capacity to diminish inflammatory variables and uphold bone health through multiple mechanisms.[10] This study utilised OPG/RANKLE to investigate the impact of probiotics on periodontal bone loss and the oral microbiota and inflammatory landscape in 150 patients with type 2 diabetes.

Methodology

This study was conducted as a randomised, controlled clinical trial on 50 individuals diagnosed with Stage I, II, or III Grade Periodontitis and 50 patients diagnosed with Stage I, II, or III Grade gingivitis who also had type 2 diabetes. The age range of the participants was 24-57 years. The patients included in this study were recruited from individuals who visited the clinic of the Periodontology and Dentistry Department at Al-Mustansiriyah University.

Patients in this Study

1. Experienced stage I, II, or III gingivitis
1. All patients are diagnosed with type 2 diabetes.[11]
2. The participants did not undergo periodontal therapy, get systemic antimicrobials, or take anti-inflammatory medicines within the three months preceding the start of the trial.
3. Did not take any systemic medicines that affect bone remodeling for at least 12 months prior to the start of the study. The patients that were chosen were randomly divided into two equal groups by tossing a coin.

Group I consisted of individuals who had type 2 diabetes and were diagnosed with stage I, II, or III grade of gingivitis. Which involved scaling, along with the application of topically administered probiotics gel.
Group II (control group): patients have type 2 diabetes with periodontitis and have not received topically applied probiotics gel.

Clinical Examination

Patients underwent clinical evaluation at the beginning of the study before undergoing scaling and root planing (SRP) and again before the use of probiotics. Subsequently, in the group with periodontitis, the root planing evaluations were conducted at 1 and 3 months using the following parameters: bleeding on probing (BP), plaque index (PI), pocket depth (PD), and clinical attachment level (CAL).

Biochemical Assessment

Samples of saliva were obtained at the beginning, after 1 month, and after 3 months to measure the levels of RANKLE and OPG using enzyme-linked immunosorbent assay (ELISA).

Sample Storage

Samples are stored at a temperature of -80˚C until they are ready for biochemical examination.

Probiotic Preparation

1. ProlacSan® Gel syringe, consists of probiotic powder and thickener that are securely sealed in a metal foil. Each syringe contains a total of 6 x 10^9 colony-forming units (CFU) of Lactobacillus brevis and plantarum.
2. To prepare, draw up a maximum of 1.2ml of distilled water into a syringe, agitate gently, and allow to sit for at least 5 minutes, ideally 15 minutes.

Probiotic Application for the Test Group

After the standard periodontal therapy (SRP):

- Use cotton rolls to isolate the desired site and ensure it is completely dry before applying the gel.
- The gel was meticulously put below the gumline into the pocket until there was an excess amount of gel visible at the gum edge. The surplus gel was then removed.

Post-treatment Instruction

Patients who were administered probiotics were advised to abstain from rinsing, eating, or drinking for a minimum of 2 hours. They were also instructed not to disturb the treated area with their tongue, finger, or toothpick, and to avoid chewing any hard or sticky food for at least 1 week. Additionally, they were advised to postpone brushing and flossing the treated site for a few days.

Elisa test Kit

The levels of OPG and RANKLE were quantified using a double sandwich ELISA technique. Enzyme-linked immunosorbent assay (ELISA) is widely regarded as the most reliable method for detecting disease-related biomarkers in clinical laboratories worldwide. Commercially available ELISA tests can be used to diagnose neurological disorders, cancers, and inflammatory diseases. Subtle variations in expression may allow for the detection of numerous biomarkers associated with sickness. The enzyme-

linked immunosorbent assay (ELISA) was derived from a radioimmunoassay (RIA) that was created by Hoffman[11]. In a conventional ELISA, antibodies are employed to immobilise antigens on an ELISA plate, which serves as a firm support made of plastic. Subsequently, an enzyme is employed to transform the substrate into a discernible signal. The signal strength is directly proportional to the concentration of the antigen.[12] Therefore, ELISA can be utilised to identify the antigen and measure its quantity. This is accomplished by sequentially introducing ELISA components onto the assay plate, followed by incubation, and subsequently analysing the obtained data. The fundamental components of an ELISA experiment include antigen, substrate, secondary antibody, enzyme-conjugated antibody, and primary antibody.[13] During ELISA, a variety of buffers are used to dilute the components, wash away any surplus chemicals, fill in any voids on the plate, and halt the substrate reaction. Several variants of ELISA have been created throughout time to boost the assay's specificity, decrease interference, and increase sensitivity. ELISA based on nanoparticles, sandwich ELISA, indirect ELISA, competitive ELISA, and direct ELISA are the five most common forms of these techniques. The binding of antigens to antibodies is the same across all ELISA formats; what varies is the sequence and number of steps.[14]

Statistical Analysis

Quantitative analysis of data using statistical methods. The data were collected and processed using the SPSS version 23.0 application, which involved tabulating, computing, and doing statistical analysis.

Results

The impact of various treatment methods on both the clinical and biochemical factors is demonstrated in tables (1), (2), (3), and (4).

Changes in Bopbleeding on probing) BP), and Plaque index (PI)

- Both groups experienced a notable and consistent decrease in (BP) and plaque index (PI) from the beginning to the end of the 3-month period.
- There were no statistically significant disparities between the two groups at the start, after 1 month, and after 3 months.

Table 1: The Mean ± Standard Deviation (SD) and p-values of Biochemical Parameters bp, PI Levels at Different Intervals for Gingivitis Group.

Biochemical Parameters		Group I(n=50) Simple Gingivitis Mean	SD	Moderate Gingivitis Mean	SD	Sever Gingivitis Mean	SD	Group II(n=25) Mean	SD
Bp	Base line	2.49	0.3	2.62	0.4	3.21	0.4	2.41	0.4
	1 month	0.89	0.4	1.14	0.4	2.09	0.5	1.02	0.5
	3 months	0.89	0.9	1.07	0.8	1.47	0.7	1.03	0.6
	P-value*			< 0.001				< 0.001	
PI	Base line	2.64	0.5	2.76	0.3	3.6	0.1	2.58	0.6
	1 month	0.53	0.1	0.68	0.3	2.01	0.2	0.7	0.1
	3 months	0.74	0.2	0.93	0.4	1.21	0.4	1.11	0.6
	P-value*			< 0.001				< 0.001	

Table 2: The Mean ± Standard Deviation (SD) and p-values of Clinical Parameters OPG, RANKLE Levels at Different Intervals for Gingivitis Group.

Clinical Parameters		Group I(n=50) Simple Gingivitis		Moderate Gingivitis		Sever Gingivitis		Group II (n=25)	
OPG	Base line	4.06	0.7	3.86	0.8	3.42	0.5	4.41	0.5
	1 month	4.64	0.6	4.44	0.7	3.75	0.5	4.57	0.7
	3 months	4.81	0.8	4.54	0.8	4.09	0.6	4.93	0.7
	P-value*			0.166				0.268	
RANKLE	Base line	75.04	9.4	82.04	8.9	93.04	8.4	93.8	9.1
	1 month	68.84	9.5	74.54	9.4	88.64	8.6	103.7	13.3
	3 months	61.93	9.9	67.98	9.7	71.8	9.1	106.2	11.9
	P-value*			0.002				0.437	

Table 3: The Mean ± Standard Deviation (SD) and p-values of Biochemical Parameters bp, PI, PPD, CAL Levels at Different Intervals for Periodontitis Group.

Clinical and Biochemical Parameters		Group I(n=50) Simple Gingivitis Mean	SD	Moderate Gingivitis Mean	SD	Sever Gingivitis Mean	SD	Group II (n=25) Mean	SD
Bp	Base line	1.89	0.2	1.94	0.3	2.67	0.2	2.41	0.4
	1 month	0.78	0.6	1.02	0.2	1.53	0.1	1.02	0.5
	3 months	0.71	0.3	0.94	0.5	1.18	0.4	1.03	0.6
	P-value*			< 0.001				< 0.001	
PI	Base line	1.80	0.3	2.31	0.2	3.24	0.4	2.58	0.6
	1 month	0.61	0.1	0.73	0.5	2.18	0.7	0.7	0.1
	3 months	0.43	0.3	0.50	0.1	1.62	0.2	1.11	0.6
	P-value*			< 0.001				< 0.001	
PPD	Base line	4.09	0.9	4.34	0.8	4.81	0.9	3.9	0.9
	1 month	2.64	0.5	3.25	0.9	3.77	0.8	2.66	0.5
	3 months	2.13	0.6	2.31	0.8	2.94	0.6	2.53	0.4
	P-value*			< 0.001				0.0034	
CAL	Base line	2.67	0.7					2.28	0.8
	1 month	1.26	0.9					1.43	0.8
	3 months	0.73	0.1					1.38	0.7
	P-value*			< 0.001				0.017	

Table 4: The Mean ± Standard Deviation (SD) and p-values of Clinical Parameters OPG, RANKLE Levels at Different Intervals for Periodontitis Group.

Clinical Parameters		Group I(n=50)						Group II(n=25)	
		Simple Periodontitis		Moderate Periodontitis		Sever Periodontitis			
OPG	Base line	4.71	0.4	4.10	0.5	3.86	0.3	4.41	0.5
	1 month	5.07	0.6	4.61	0.7	3.97	0.3	4.57	0.7
	3 months	5.18	0.2	4.83	0.3	4.08	0.7	4.93	0.7
	P-value*			0.166				0.268	
RANKLE	Base line	81.14	7.4	87.19	9.4	95.10	7.2	93.8	9.1
	1 month	72.07	7.8	81.64	9.6	86.22	8.3	103.7	13.3
	3 months	60.83	7.1	78.88	9.8	82.30	7.8	106.2	11.9
	P-value*			0.001				0.382”	

Changes in Pocket Depth (PD) and Clinical Attachment Level (CAL)

- Both groups experienced a notable and consistent drop in PPD (probing pocket depth) and CAL (clinical attachment level) from the beginning to the 3-month mark.
- There were no statistically significant variations between the two groups at the start and after 1 month. There was a statistically significant difference observed between the two groups after 3 months. Regarding the interaction between time and treatment group, there was a significant difference in the improvement of results observed in group I compared to group II over time.

Changes in OPG Level

- Group I: The OPG level showed a slight rise over time from baseline to 3 months, with a p-value of 0.166. However, it has decreased within the three subgroups of this group. There was a small variation between the first measurement and the one taken after 3 months (p=0.161). The baseline was somewhat higher than 1 month, but the difference was not statistically significant (p=0.616).
- Group II: The level of OPG showed an insignificant rise during the study period.
- There were no statistically significant disparities between the two groups at the beginning, after 1 month, and after 3 months.

Changes in RANKLE Level

- Group I: There was a notable reduction in the RANKLE level over time, namely from baseline to 3 months, with a p-value of 0.002. However, among subgroups of this group, the RANKLE level showed an increase. A substantial statistical difference was observed between the baseline and 3-month time points, as well as between the 1-month and 3-month time points. However, the baseline measurement was not substantially greater than the measurement taken at 1 month (p=0.745).
- Group II: The RANKLE level showed a statistically insignificant increase during the study period, with values of 93.8 ± 9.1 at baseline, 103.7 ± 13.3 at 1 month, and 106.2 ± 11.9 at 3 months (p=0.374).

There were no statistically significant differences between the two groups at baseline and after 1 month. At the 3-month mark, the RANKLE level in group I was notably lower compared to group II (p=0.029). Regarding the interaction between time and treatment group, there were significantly improved results in terms of RANKLE level in group I compared to group II over time (p = 0.028).

Discussion

Periodontitis immunological disruption is characterised by persistent and chronic inflammation that creates an environment conducive to the growth of harmful bacteria.[15,16] The significance of tumour necrosis factor is clearly apparent in the inflammatory process and immune cell response that leads to bone loss in periodontitis.[17] The onset and advancement of periodontal inflammation may be attributed to an inadequate or unfavourable reaction of the anti-inflammatory cytokines.[18] Tumour necrosis factor has demonstrated significant efficacy in regulating the secretion of pro-inflammatory cytokines, including IL-1β, TNF-α, and IL-6. It also mitigates the detrimental consequences of inflammation, such as bone resorption, while promoting the healing process of periodontal tissues.[19] Osteoprotegerin (OPG) plays a crucial role in the development of periodontal disease by acting as a receptor for RANKL, hence inhibiting the differentiation of osteoclasts. The RANKL-OPG system plays a vital role in controlling the formation of osteoclasts and the breakdown of bone in both normal and abnormal situations.[20] The decreased ratio of RANKL/OPG in areas with destructive periodontal activity following periodontal treatment provides evidence for the utilisation of these molecules in the diagnosis, monitoring, and treatment of periodontal disease.[21] In the 2017 classification, the diagnosis of periodontitis is made using a multi-dimensional categorisation based on stages and grades. This approach allows for a more tailored and patient-centered treatment, taking into account the patient’s medical history.[22] This study focused on the inclusion of Stage I, II, and III periodontitis cases.

Engage in academic pursuits. If detected early, the condition has a highly favourable prognosis. However, if treatment is delayed, it can lead to significant damage to the teeth. Non-surgical periodontal therapy is the primary approach in treating periodontal disease. Its goal is to

eliminate the cause of the illness, halt its progression, and promote the repair of the root surface to a biologically acceptable state. Not only is it the primary method of treating periodontal disease, but it also effectively restores tissue health.[23] .Probiotics were administered locally as an additional treatment with regular periodontal therapy. It can be utilised as a standalone treatment or as an additional therapy, not only to avoid infections but also to interfere with the microbial pathways that lead to inflammatory immunological illnesses. The simplicity of administering probiotics and the absence of any negative effects recorded in scientific literature have generated growing interest among researchers in using this preventive method for many diseases, such as periodontal disorders.Probiotics can enhance alveolar bone and attachment level, as well as improve microbiological and immunological results when used to treat periodontitis in animal models.[10,24,25] This study utilised a gel formulation of probiotics comprising Lactobacillus brevis and plantarum. The gel was administered using a syringe to ensure precise delivery to the pocket's depth, maximising its effectiveness. Particular strains of probiotic bacteria, such as L. plantarum GOS42, have the potential to be used in oral care products to decrease inflammation of the gums. These strains have been found to reduce the release of inflammatory substances by human monocytes when exposed to bacterial LPS. These anti-inflammatory effects are strong and not significantly affected by the viability of the bacteria.[26] A recent study conducted in 2022 provided data on the use of a locally applied mucoadhesive gel containing Lactobacillus probiotic strains for the prevention and treatment of periodontitis.[27] The current study excluded individuals who smoke, are pregnant, lactating, or have medical conditions that could significantly increase the risk of periodontal disease. In addition, patients who had undergone periodontal therapy received antimicrobials or anti-inflammatory drugs within the past 3 months and had taken bone remodelling drugs within the 12 months prior to the study were excluded. This was done to ensure that the research results are reliable and accurate, in accordance with the guidelines for periodontal therapy.[28] The current study utilised clinical and biochemical evaluation to assess inflammatory and bone turnover biomarkers by collecting and analysing gingival crevicular fluid from individuals with type 2 diabetes. This approach can be seen as a supplementary method to the conventional techniques of periodontal diagnosis, as it plays a crucial role in identifying individuals who are at risk for future periodontal issues.

The breakdown also includes the assessment of therapy results.[29-31] There was a statistically significant decrease in plaque and bleeding on probing (BP) across the trial period, from baseline to 3 months, in both groups. At baseline, there were no statistically significant differences between the two groups. However, the probiotics group showed improved results compared to the control group, but these changes were not statistically significant. These results align with the findings of many studies that have shown a notable enhancement in PI (periodontal index) and BP (blood pressure) following probiotic treatment.[29,32,33] A separate study demonstrated that regular intake of probiotic milk can alleviate the symptoms of gingival inflammation caused by plaque in individuals with a higher plaque score.[34-38] Another recent study indicated that the administration of probiotics did not appear to be advantageous in preventing plaque buildup, since it could be influenced by inadequate dental hygiene.[39] The administration of probiotic tablets comprising L. plantarum, L. brevis, and P. acidilactici did not result in any substantial alterations in the average blood pressure. Furthermore, the test group saw a substantial decrease in the frequency of locations with elevated blood pressure and severe inflammation, as well as a noteworthy microbiological influence.[40]

The observed clinical outcomes can be attributed to mechanical removal of debris, patient motivation, and instruction on oral hygiene measures offered to both groups.[41] In this investigation, the assessments of pocket depth and clinical attachment loss showed a considerable and consistent decline with time, from the initial measurement to the 3-month mark, in both groups. Although there were no significant changes between the two groups at the beginning and after one month, a significant difference was observed between the two groups after three months. Additionally, the probiotic group showed better results in terms of PPD and CAL over time compared to the control group. These results are in line with multiple studies that have demonstrated a significant clinical improvement linked to a reduction in PPD (probing pocket depth) and an increase in CAL (clinical attachment level) gain.[32,33,37-39] Moreover, there was an increase in the amount of gingival crevicular fluid and a decrease in the presence of periodontopathogens and pro-inflammatory markers in individuals with periodontal disease.[38] Another study reported a statistically significant decrease in pocket depth and an increase in attachment in moderate and deep pockets ($p < 0.05$).[42] The use of a dietary supplement in the form of a suspension containing L. salivarius SGL03 has been indicated to potentially reduce pocket depth, even if there were no observed changes in other clinical parameters or the quantity of bacteria in supragingival plaque.[43] Furthermore, the ingestion of L. reuteri Prodentis orally resulted in enhanced immediate clinical results, such as reduced bleeding on probing and decreased pocket probing depths, in individuals with type 1 diabetes and initial-to-moderate chronic periodontitis.[33] Another study that utilised probiotics demonstrated a significant increase in the number of periodontal pockets converting from ≥4 mm at the beginning of the study to ≤3 mm after 24 weeks. Additionally, there were fewer locations that required surgical intervention (4 ± 4% versus 8 ± 6%).[44] The biochemical test of the probiotic group showed a considerable drop in the RANKLE level throughout time, specifically from baseline to 3 months. There was a statistically significant difference between the baseline

and 3-month measurements. However, the control group showed a negligible increase over the duration of the trial. There were no statistically significant differences between the two groups at the beginning of the study in terms of RANKLE level. However, subgroup I showed significantly better results in RANKLE level compared to subgroup III, especially after 3 months (p=0.029). The findings of this study support the use of probiotic supplementation with L. rhamnosus and L. acidophilus, which had a notable impact on the severity of apical periodontitis in rats. This demonstrates the anti-inflammatory properties of probiotics in the development of apical periodontitis, as evidenced by the significantly higher IL-10 immunolabeling in the groups receiving probiotics.[45] A 2022 investigation utilizing a probiotic complex found a substantial increase in IL-10 levels in the group receiving probiotics.[46] There is a significant discrepancy among numerous research studies that have uncovered multiple variances in the OPG level. Extensive research, including both animal and human trials, is necessary to establish the probiotic effect on the RANKL/RANK/OPG pathway. The expression level of OPG after non-surgical periodontal therapy did not exhibit any significant alterations when compared to healthy subjects.[47] In addition, another study indicated that probiotics have the potential to decrease inflammatory markers such as TNF-α and IL-1β, while simultaneously increasing the production of bone OPG.[48] An experiment revealed that treatment with either Lactobacillus para or the Lactobacillus mix alters the immune response in bone by reducing cytokine levels and increasing OPG expression.[49] During a study on periodontal disease, it was found that the addition of probiotics did not result in a decrease in the expression of RANKL. However, the supplementation of OPG considerably increased by 50 units.[50] However, in another case, there was a decline in the amount of RANKL while the level of OPG remained constant.[46] Out of all the studies, this particular study. It was noted that the OPG level initially revealed no significant differences between the two groups at the beginning of the trial. However, subgroup I and subgroup III demonstrated a consistent but insignificant increase in OPG levels throughout the study period, with subgroup III showing better outcomes. The discrepancy in results can be attributed to variations in the species utilised, as higher amounts of OPG were often observed with specific Lactobacillus species compared to others, as well as the proportion contributed by each species.

Conclusions

- The application of a probiotic gel as an additional treatment showed an inflammatory effect by significantly increasing the expression of tumor necrosis factor in patients with stage I, II, and III periodontitis.
- The application of probiotic gel significantly enhanced bone formation by reducing the expression of OPG after periodontal therapy.
- Using probiotic gel as an additional treatment resulted in a significant decrease in probing pocket depth and an increase in attachment level during non-surgical treatment of stage I, II, and III periodontitis.

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Conflict of Interest

Nil.

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Oral immunity - implant- oral medicine.

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