https://doi.org/10.26682/sjuod.2022.25.2.35
Journal of University of Duhok., Vol. 25, No.2(Pure and Engineering Sciences), Pp 380-388, 2022

PROBIOTIC BACTERIAL EFFECTS ON THE TREATMENT OF CHRONIC PERIODONTITIS PATIENTS: A DOUBLE-BLIND STUDY

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(Received: October 2, 2022; Accepted for Publication: November 8, 2022)

ABSTRACT

Background: Technology of Probiotics indicates an approach discovery on sustaining oral health via helpful natural bacteria, which are usually found in the oral cavity, to make a good protection against those bacteria which are supposed to be destructive to gingival tissue and teeth.

Aims: This article goal was to assess the influence of probiotics that utilized as an adjunctive method to scaling and root planning (SRP) on measurements of the periodontium and matrix metalloproteinase-8 (MMP-8) and interleukin-6 (IL-6) levels in gingival crevicular fluid (GCF) of patients with chronic periodontitis.

Material and Methods: Forty persons aged between 35 and 55 years had chronic periodontitis were healthy systemically, and they had moderate to severe chronic periodontitis. Two groups of participants were created randomly double blindly. Both groups were assessed thirty days after beginning the periodontal treatment: the first group (N = 20) received SRP with a placebo, whereas the second group (N = 20) received SRP with probiotic capsules.

Results and Conclusion: The study results showed that periodontal indicators improved in both groups, particularly those with probiotic treatment. As well, they had a tendency for improvement but they were not statistically significant regarding the immunologic responses in those treated with probiotics as well as to SRP therapy than in patients treated with SRP alone.

***KEYWORDS*:** Probiotics, GCF/MMP-8, GCF/IL-6, Chronic periodontitis, SRP.

INTRODUCTION

Periodontitis is an infection produced by special microbes which exist in biofilms that make inflammation of the teeth supportive tissues, results in bone and periodontal ligament damages (Sharma *et al*., 2017).

The most common form of periodontal status is chronic periodontitis which leads to periodontal destruction, recurrent pain, leading to tooth loosen. The periodontitis risk factors occurred by a collection of environmental, acquired and inherited reasons. Thus, accumulation of bacterial plaques and host immune response defects play an important role for the emergence of chronic periodontitis (Alshareef *et al*., 2020).

Although the Scaling and root planning (SRP) is the golden standard management of periodontal inflammation, the managed sites will be re- colonized with microorganisms analogous to that existed previously, this will specify restrictions on the use of SRP in the management of periodontal inflammation (Mombelli, 2018).

There are numerous additional therapies utilized to improve periodontal consequences rather than scaling and root planning comprises antimicrobial agents, photodynamic management, and usage of probiotics (Ikram *et al.*, 2019).

Probiotics are defined as "live microorganisms which when administered in adequate amounts confer a health benefit on the host. Therefore, researchers have proposed that probiotics may prevent gastrointestinal disorders by maintaining homeostasis of the gut microbiome (Laleman *et al*., 2015).

Finding the mechanisms of attachment and stability and their beneficial effects on the immune system can be useful in identifying and increasing the therapeutic effects of probiotics.

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In this review, the functional mechanisms of probiotics were comprehensively investigated. Relevant articles were searched in scientific sources, documents, and databases, including PubMed, NCBI, Bactibace, OptiBac, and Bagel4. The most important functional mechanisms of probiotics and their effects on strengthening the epithelial barrier, competitive inhibition of pathogenic microorganisms, production of antimicrobials, binding and interaction with the host, and regulatory effects on the immune system were discussed (Salas-Jara *et al.,* 2016).

It should be noted that the probiotic effects of different strains are not the same. Therefore, the health benefits attributed to one strain, also in one species, may not necessarily apply to another strain. A specific strain of probiotics shows benefit of the health including following genus and species: *Lactobacillus, Leuconostoc, Bacillus, Escherichia coli, Bifidobacterium, Saccharomyces, Enterococcus, Streptococcus, and Pediococcus* (Fijan, 2014).

The *Lactobacillaceae* family includes the genus *Lactobacillus*, which is a Gram-positive aerobic bacterium that is also prefers to grow in an anaerobic environment. When periodontal inflammation is present, Lactobacillus is even more beneficial (Elavarasu *et al*., 2012).

The previous studies have shown that the considerable decline in gingival index after thirty days in patients managed with probiotics in comparison with the patients managed without using probiotics sheds light on the role of probiotic microbes in the gingivitis owing to its antimicrobial action in contrast to pathogenic microorganisms (Shimauchi *et al.,* 2008; Morales *et al.,* 2018).

The periodontium destruction is principally attributed to enzymes that are distributed during the process of the inflammation. The matrix metalloproteinases (MMPs) are a set of particular enzymes that act a substantial effective role in mutually physiological and pathological processes (Nędzi-Góra *et al*., 2017).

Matrix metalloproteinase-8 (MMP-8) is one of the MMPs that exists in high levels in gingival crevicular fluid (GCF), saliva and in gingival tissue of patients with periodontal inflammation, and it might be a marker of the existence of periodontitis (Javed *et al.,* 2014).

Interleukin-6 is an additional indicator, that is pro-inflammatory cytokines act as substantial role in resorption of the bone in chronic inflammation. The immune system is strongly linked to the maintenance of healthy bone. Inflammatory cytokines, specifically, are crucial to skeletal homeostasis and any dysregulation can result in detrimental health complications. Interleukins, such as interleukin 6 (IL-6), act as osteoclast differentiation modulators, must be carefully monitored and regulated. IL-6 encourages osteoclastogenesis when bound to progenitors and can cause excessive osteoclastic activity and osteolysis when overly abundant (Scheller *et al*., 2011).

The study's objectives: 1. To evaluate clinical and immunological effect of non-surgical periodontal management (scaling and root planning) in patients have chronic periodontitis with and without the use of probiotics. 2.To estimate the level of MMP-8 and IL-6 in GCF of patients have chronic periodontitis prior and after nonsurgical periodontal management with and without the uses of probiotics.

MATERIALS AND METHODS

Participants and characteristic:

The study was considered a double blind randomized clinical study and the proposal was accepted by the ethical committee, College of Dentistry, Duhok University. The individuals in this study were sourced from the periodontal department in Dentistry College Dental Clinics in Duhok University. Forty persons aged between 35 and 55 years have chronic periodontitis were healthy systemically. The 40 participants in the research had moderate to severe chronic periodontitis. Two groups of participants were created. Both groups were assessed thirty days after beginning the periodontal treatment: the first group (N = 20) received SRP with a placebo, whereas the second group (N = 20) received SRP with probiotic capsules.

Probiotic capsules prescription:

The probiotic capsules (Flora GX., USA) were given once a day to the second group after SRP. Each probiotic capsule contains a billion of CFU (colony forming unit) and comprises 9 probiotics bacterial of *Lactobacillus* species which comprises of: *Lactobacillus plantarum, Lactobacillus paracasei, Lactobacillus bulgaricus, Lactobacillus casei, Lactobacillus acidophilus, Lactobacillus brevis, Bifidobacterium lactis* and *Lactobacillus salivarius.*

Periodontal therapy and clinical examination:

The periodontal measurements comprised plaque index (PI), gingival index (GI), periodontal pocket depth (PPD), and clinical attachment loss (CAL) (Rheu et al., 2011), were measured for all persons prior and following periodontal therapy for both groups. All patients took nonsurgical periodontal treatment, which included, scaling and root planning, polishing, and chemical wash. To ensure the removal of supra and subgingival calculus, precise root planning, and managing of deep pockets utilizing an ultrasonic scaler and manual instruments, the scaling and root planning were carried out on two different appointments (scalers and Gracey curettes). All participants were advised to follow oral hygiene rules to keep plaque under control. Following thirty days, both groups were assessed in order to evaluate the clinical measurements and collect gingival crevicular fluid specimens.

GCF sample collection and MMP-8 & IL-6 assay:

The entire deposits of supragingival were carefully removed prior to collection of GCF without causing any damage to the gingival tissue. Afterwards, to prevent contamination of salivary, the region was isolated, then the right and left maxillary quadrant pockets of 3 µL of GCF were obtained by utilizing micropipettes and capillary tubes. A baseline and after1 month of periodontal treatment the samples of GCF were taken from all participants.

The samples of GCF were diluted in 200 µL of phosphate buffered solution and placed in a 1.5 mL Eppendorf tube. This tube was then stored at -80°C until it was used. The MMP-8 and IL-6 levels were estimated by using human MMP-8 ELISA kit (MMP-8 BT- Lab kit, China) and IL-6 ELISA kit (IL-6 BT- Lab kit, China), according to manufacturer instructions.

Briefly, 50 µL of standard was added to standard well and 40 µL of sample to sample well, then 10 µL of anti-MMP-8 / anti-IL-6 antibody was added to sample wells, then 50 µL of streptavidin-HRP was added to sample and standard wells and incubated 60 minutes at 37 C. Afterwards washing 5 times with washing buffer, we added 50 µL of substrate A and 50 µL of substrate B to each well then incubated 10 minutes at 37 C. Following that the use of a 50 µL stop solution to stop the reaction, then a MICROPLATE reader was used to read the optical density readings at 450 nm.

Statistical analysis

The general information of the patients in both placebo and probiotics groups was presented in mean and standard deviation (SD). The levels of clinical parameters and MMP-8 and IL-6 were determined in mean and standard deviation (SD) . An independent t-test was performed to examine the comparisons of clinical parameters, IL-6 and MMP-8 between study groups at follow-up. The comparisons of pre and post of clinical parameters, IL-6 and MMP-8 in placebo and probiotics were tested in a paired t-test. The p-value less than 0.05 indicated a significant difference. The statistical analyses were carried out using JMP Pro 14.3.0. By using the Microsoft Word application (version 13).

RESULTS

Periodontal Parameters Evaluation

Mean, standard deviation, and p-values were used to express the descriptive statistics of periodontal parameters for both groups. (Table 1, 2) at the baselines, the periodontal parameters mean for placebo group was PI =1.85 ±SD (0.35), GI= 2.16 ±SD (0.33), PPD= 5.11±SD (0.33) and CAL= 3.43 ±SD (0.43). whereas for probiotics group, the mean was PI= 1.89 ±SD (0.22), GI= 2.09 ±SD (0.11), PPD= 5.28 ±SD (0.29) and CAL= 3.62 ±SD (0.39). periodontal therapy with or without probiotics was completed thirty days later, the periodontal parameters mean of placebo group was PI= 1.29 ±SD (0.14), GI= 1.47 ±SD (0.18), PPD= 3.96 ±SD (0.22) and CAL= 2.38 ±SD (0.35). whereas for probiotics group was PI= 1.21 ±SD (0.11), GI= 1.36 ±SD (0.15), PPD= 3.75 ±SD (0.39) and CAL= 2.32 ±SD (0.34).

Table (1): Comparisons of general and clinical parameters of study groups at baselines and follow up (after 30 days).

Characteristics and Clinical parameters	**Study groups Mean ±(SD) at baseline**		**p-value (two-sided)**
	Placebo patients (n=20)	**Probiotics patients (n=20)**	
Age (year)	41.3 ± 6.02	40.75 ± 6.48	0.7824[a]
Gender no (%)			0.2059[b]
Male	8 (40%)	12 (60%)	
Female	12 (60%)	8 (40%)	
PI	1.85 ± 0.35	1.89 ± 0.22	0.7074[a]
GI	2.16 ± 0.33	2.09 ± 0.11	0.4491[a]
PPD	5.11 ± 0.33	5.28 ± 0.29	0.1051[a]
CAL	3.+43 ± 0.43	3.62 ± 0.39	0.1718[a]
Clinical parameters	**Study groups Mean ±(SD) at follow up (after 30 days)**		**p-value (two-sided)**
	Placebo patients (n=20)	**Probiotics patients (n=20)**	
PI	1.29 ± 0.14	1.21 ± 0.11	0.0579
GI	1.47 ± 0.18	1.36 ± 0.15	**0.0417**
PPD	3.96 ± 0.22	3.75 ± 0.39	**0.0489**
CAL	2.38 ± 0.35	2.32 ± 0.34	0.5794

* Abbreviations: GI, gingival index; CAL, clinical attachment loss; PPD, Periodontal pocket depth; PI, plaque index; SD, standard deviation.
* For statistical analysis, a Pearson chi-squared test and an independent t-test were also used.

Table (2): Pre and post Statistical comparisons of clinical parameters in placebo and probiotics.

Clinical parameters	Placebo Mean ± (SD)			
	Baseline	**Follow-up (After 30 days)**	**Mean diff (95% CI)**	**p-value**
PI	1.85 ± (0.35)	1.29 ± (0.14)	-0.56 (-0.68 to -0.44)	**<0.0001**
GI	2.16 ± (0.33)	1.47 ± (0.18)	-0.69 (-0.80 to -0.58)	**<0.0001**
PPD	5.11 ± (0.33)	3.96 ± (0.22)	-1.15 (-1.24 to -1.07)	**<0.0001**
CAL	3.43 ± (0.43)	2.38 ± (0.35)	-1.01 (-1.08 to -0.95)	**<0.0001**
	Probiotics Mean ± (SD)			
PI	1.89 ± (0.22)	1.21 ± (0.11)	-0.68 (-0.75 to -0.60)	**<0.0001**
GI	2.09 ± (0.11)	1.36 ± (0.15)	-0.75 (-0.80 to -0.69)	**<0.0001**
PPD	5.28 ± (0.29)	3.75 ± (0.39)	-1.57 (-1.63 to -1.52)	**<0.0001**
CAL	3.62 ± (0.39)	2.32 ± (0.34)	-1.3 (-1.35 to -1.25)	**<0.0001**

*Abbreviations: GI, gingival index; CAL, clinical attachment loss; PPD, Periodontal pocket depth; PI, plaque index; SD, standard deviation.
* For statistical analysis, paired t-test was used.
*The p-value < 0.01 (was considered highly significant).

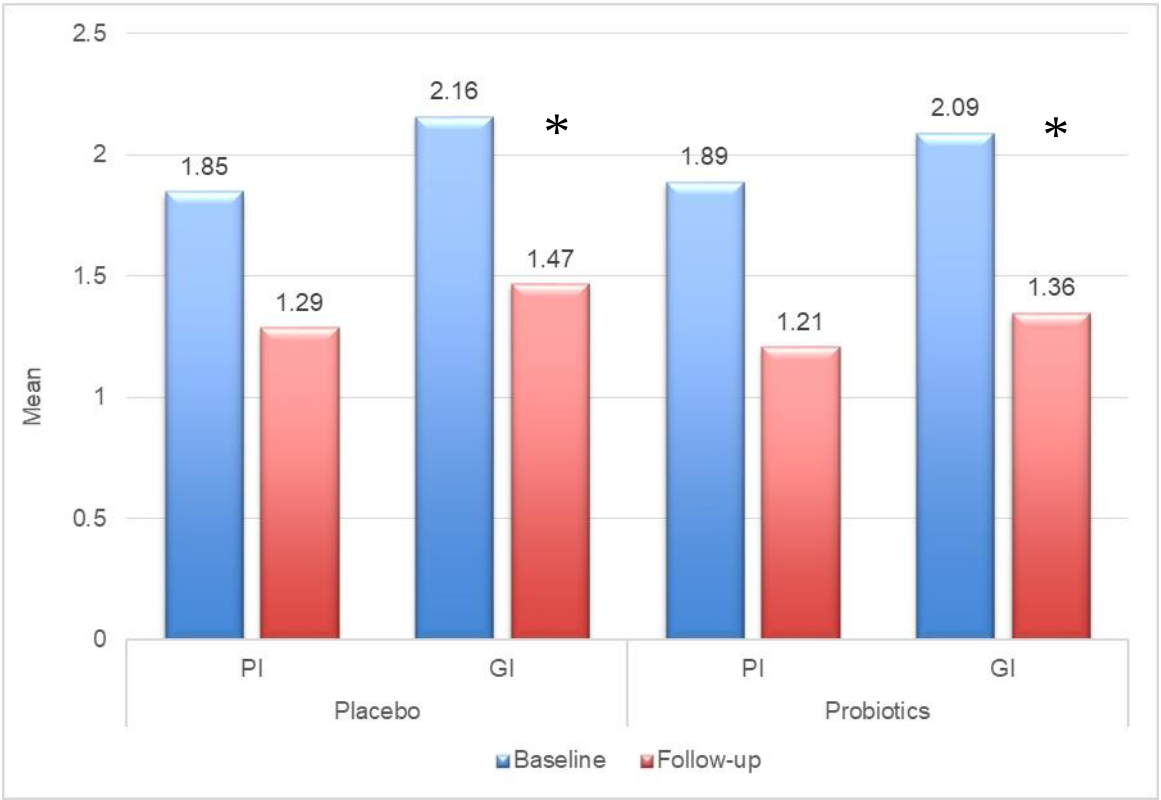

Fig. (1): An illustration of the plaque index (PI) and gingival index (GI) means for the two groups under study

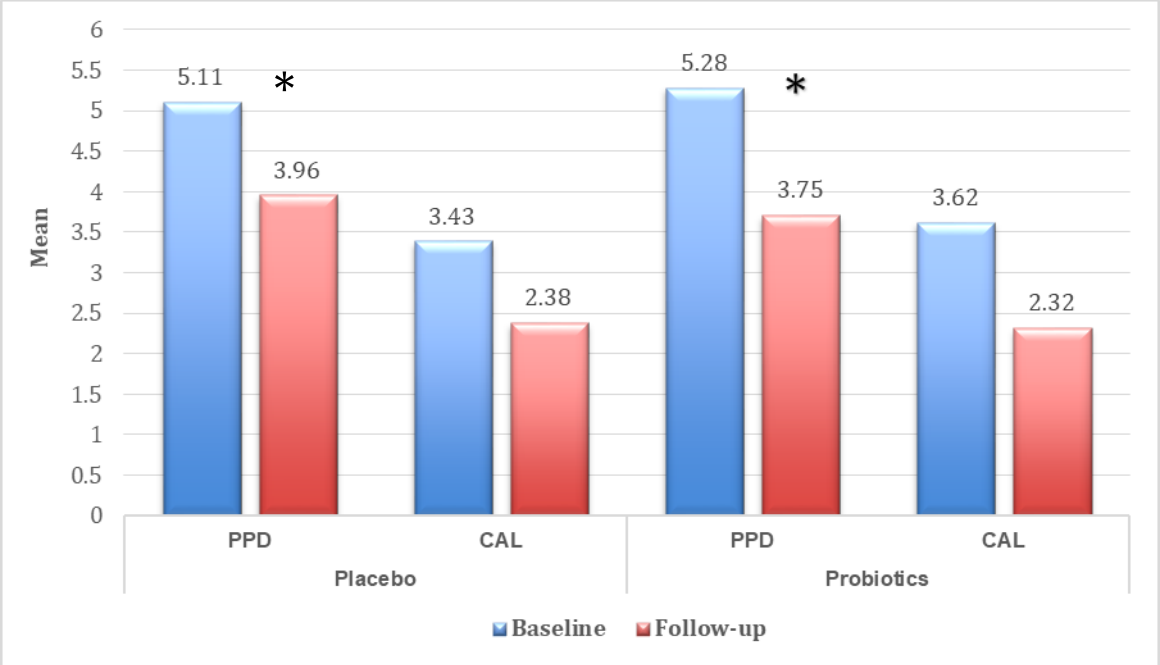

Fig. (2): An illustration of the clinical attachment loss (CAL) and periodontal pocket depth (PPD) means for the two groups under study.

Evaluating of MMP-8 and IL-6 Levels

GCF/MMP-8 and GCF/IL-6 levels were significantly declined thirty days later among periodontal therapy individuals treated only by scaling and root planning compared to the baseline, however, the number of patients receiving probiotics and scaling and root planning therapy had a high significantly declined rate. Additionally, at baseline and thirty days following scaling and root planning therapy, there were no statistically significant variations in the GCF/MMP-8 levels and GCF/IL-6 levels between placebo group and probiotics group (Table 3).

Table (3): t-Test statistical Comparisons of pre and post treatment levels of IL-6 and MMP-8 in placebo and probiotics.

Clinical parameters	**Placebo Mean ± (SD)**			
	Baseline	**Follow-up (After 30 days)**	**Mean diff (95% CI)**	**p-value**
IL-6 (pg/ml)	34.65 ± (4.77)	33.33 ± (4.64)	-1.32 (-1.57 to -1.07)	**<0.0001**
MMP-8	36.01 ± (1.88)	34.15 ± (2.32)	-1.86 (-2.59 to -1.13)	**0.0003**
Clinical parameters	**Probiotics Mean ± (SD)**			
IL-6 (pg/ml)	36.02 ± (4.60)	33.22 ± (4.68)	-2.79 (-3.06 to -2.53)	**<0.0001**
MMP-8	37.02 ± (0.51)	33.62 ± (1.10)	-3.40 (-3.81 to -2.99)	**<0.0001**

*Abbreviations: GCF-MMP8, gingival crevicular fluid-matrix metalloproteinase-8; GCF-IL-6, gingival crevicular fluid- interleukin-6; SD, standard deviation.
*The p-value < 0.001 (was considered highly significant).
*Paired t-test was performed for statistical analyses.

DISCUSSION

Periodontitis is a disease that has many criteria which includes inflammation of soft and hard periodontal tissues, colonization of bacteria, and adaptive immune responses. Periodontal disease is treated with nonsurgical methods as well as surgical therapy, that focuses primarily on mechanical debridement and, in certain circumstances, is combined with antibiotics. These therapeutic approaches attempted to eradicate all periodontal infections that had colonized and spread throughout the periodontal tissues. A new treatment approach for treating periodontal disease was required due to resistance of antibiotics and the frequent recolonization of treated regions with pathogenic microorganisms (Gupta, 2011).

Probiotics are a variety of beneficial bacterial strains that offer the host several health advantages when properly administered. Probiotics have been suggested to have a number of effects, including murdering or inhibition of growth of pathogenic bacteria, change in the proliferation and death of the cell, anti-inflammatory cytokines elevation, and pro inflammatory decline have been the cause of changing in the immunity of the host. According to prior research on probiotics as an adjunctive treatment, newly added in the periodontics field, the recent study intended to assess the probiotic effects as an adjunctive therapy to nonsurgical periodontics management on clinical periodontal parameters in addition to the gingival crevicular fluid level of MMP-8 and IL-6 in patients with chronic periodontitis (Geier *et al.,* 2007).

Our study's aim was to assess the clinical parameters and immunological reactions to periodontal treatment with and without probiotics use; depending on previous research, (Ince *et al*., 2015), that evaluated the probiotics influence in conjunction with nonsurgical periodontal treatment on some of inflammatory cytokine's levels.

In the current investigation, all clinical indicators (PI, GI, PD, and CAL) significantly improved thirty days following non-surgical management in both groups, whether probiotics were used or not. These results concur with these researches (Galofré *et al.,* 2018; Morales *et al*., 2018; and Alshareef *et al*., 2020). Nevertheless, the clinical indicators did not significantly improve after thirty days of nonsurgical periodontal management in patients with chronic periodontitis. These findings disagree with some earlier papers (İnce *et al*., 2015). To be more specific, the study's limited evaluation period could be the cause for the non-significant improvement in clinical indicators (PI and CAL).

Statistical analysis of PPD and GI in two groups at thirty days revealed a significant decline in probiotic-treated patients. Previous studies provided support for this notification (Galofré *et al*., 2018; Kumar *et al*., 2021) Probiotic bacteria play a crucial role in reducing infection because of their antimicrobic action against pathogenic bacteria, as seen by the significant reduction in PPD and GI after thirty days in probiotic-treated patients compared to those who did not use probiotics. According to earlier research (Poutahidis *et al.*,2013) probiotics raise the oxytocin synthesis, that can speed up the process of healing and also probiotics have abilities to prevent periodontal inflammation. The fact that probiotics have been investigated to perform a protective effect in the barrier of gingival epithelial by sustaining expression of protein and inhibiting apoptosis of mucous membrane could be another explanation for the improvement in the measurement of the periodontal pocket depth (Mennigen *et al.,* 2009).

MMP-8 and IL-6, the two inflammatory proteases that play a part in the etiology of periodontitis and the change from healthy periodontium to periodontal inflammation, are mostly produced by neutrophils. At baseline and later thirty days of periodontal management the gingival crevicular fluid levels of matrix metalloproteinases 8 and interleukin 6 were evaluated in both test groups. Probiotics enhance and modify the oral mucosa immune system by decreasing the pro-inflammatory cytokines production via NFkB pathway activity and rising the anti-inflammatory cytokines production, according to a previous study that provides additional support for the current study (Koduganti *et al.,* 2011). Additionally, it could be described by the test product's Lactobacilli strain's microbial antagonistic relationship with P. gingivalis, as explained by (Van Essche *et al*., 2013), who demonstrated how lactic acid

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inhibits the other bacterial strains growth by generating a large quantity of organic acids.

CONCLUSION

The current study results show that periodontal indicators improved in both groups, particularly those who received probiotic treatment. Although such improvement in the GCF/MMP-8 and GCF/IL-6 levels in patients treated with probiotics and scaling and root planning and those only treated with scaling and root planning were statistically non-significant. To better understand the clinical, microbial and immunological reactions to probiotics as an additional therapy to nonsurgical periodontal management, more study on a huge population with extended period of follow-up and using different probiotic strains and administration methods is required.

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