EGYPTIAN DENTAL JOURNAL

VOL. 63, 1437:1447, APRIL, 2017

I.S.S.N 0070-9484

ORAL MEDICINE, X-RAY, ORAL BIOLOGY AND ORAL PATHOLOGY

www.eda-egypt.org • Codex : 36/1704

THE IMPACT OF OMEGA-3 FATTY ACIDS COMBINED WITH INITIAL PERIODONTAL THERAPY ON SALIVARY VISFATIN AND TNF-α LEVELS IN CHRONIC PERIODONTITIS PATIENTS

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ABSTRACT

Recent investigations have demonstrated the positive therapeutic effects of omega-3 polyunsaturated fatty acids (PUFAs) on several chronic inflammatory diseases such as rheumatoid arthritis, chronic inflammatory bowel diseases and periodontitis. The objective of the present study was to evaluate the impact of omega-3 PUFAs in conjunction with initial periodontal therapy (IPT) on periodontal clinical parameters and salivary markers in patients with chronic periodontitis. Thirty four systemically healthy individuals with advanced chronic periodontitis were enrolled and randomly assigned into two groups. The control group (IPT, n=17) was treated with IPT alone whereas the test group (IPT+omega-3, n=17) was treated with IPT and daily dietary supplementation of omega-3 PUFAs (in the form of 2 grams of fish oil capsules per day) for three months. Clinical parameters were recorded at baseline and 3 months following therapy for both groups. Saliva samples were collected at the same time points and analyzed for visfatin and tumor necrosis factor-α (TNF-α). After 3 months, clinical periodontal parameters of advanced chronic periodontitis were significantly improved in both groups. The omega-3 group showed significant greater pocket depth (PD) reduction and clinical attachment (CAL) gain compared to the control group after therapy. Salivary TNF-α levels showed a statistically significant decrease in the omega-3 group at 3 months compared to the control group. Salivary visfatin levels were reduced significantly at 3 months in both groups without any significant variation. It was concluded that dietary supplementation with omega-3 PUFAs could be a potential viable adjunct to IPT that significantly improves all periodontal parameters and reduces salivary visfatin and TNF-α levels in advanced chronic periodontitis. Interestingly, salivary visfatin could be a useful periodontal biomarker to monitor responses to periodontal therapy.

KEY WORDS: Omega-3 fatty acids, initial periodontal therapy, chronic periodontitis, visfatin, saliva.

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INTRODUCTION

Chronic periodontitis is a local slowly progressing inflammatory disease affecting the periodontium and primarily initiated by specific anaerobic bacteria such as *Porphyromonas gingivalis*, *Tanneralla forsythia* and *Treponema denticola* which are collectively named ''the red complex''.[1] Most of the periodontal tissue destruction results from the exaggerated host response to infection and not by the direct effect of periodontopathogens.[2] The host response to these pathogens result in the release of a bunch of pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1), IL-6 and prostaglandin-E2 (PGE2). The aforementioned cytokines could activate several matrix metalloproteinases (MMPs) which result in extracellular matrix degradation of the periodontal tissues and stimulate bone resorption.[3]

A variety of locally and systemically delivered pharmaceuticals have been assessed as host modulation therapeutics (HMTs) in the treatment of periodontal disease such as non-steroidal anti-inflammatory drugs, tetracyclines, bisphosphonates and growth factors.[4] To date, dietary supplementation of omega-3 polyunsaturated fatty acids (PUFAs) has emerged with positive effects on systemic inflammation and proposed as a promising adjunctive HMT for periodontal disease.[5] Omega-3 PUFAs are naturally present in reasonably high concentrations in fish oil.[6] Two main classes have been described till now: eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). It was revealed that both EPA and DHA have anti-inflammatory effects in several diseases including periodontitis.[7] EPA and DHA are substrates for cyclooxygenase and lipoxygenase pathways with reverse actions compared to arachidonic acid, which is an omega-6 PUFA responsible for the synthesis of pro-inflammatory mediators.[8] Based upon the structural similarity, there is a competition between omega-6 PUFA and omega-3 PUFA members balancing the anti- or pro-inflammatory function. If the equilibrium shifts in favor of omega-3 PUFAs, the synthesis of pro-inflammatory products including leukotriene- B4 (LT- B4) and PGE2 are down-regulated.[9] Thus, omega-3 PUFAs could resolve inflammatory reactions via this mechanism. Omega-3 PUFAs are also substrates for generation of a novel series of bioactive lipid mediators known as resolvins and protectins that show potent protective actions in several inflammatory diseases including periodontitis.[10] The effects of omega-3 PUFAs have been investigated in a variety of experimental animal models.[11] In humans, dietary supplementation with omega-3 PUFAs has the likelihood to yield a considerable clinical outcome and pharmacologically interfere with the inflammatory cascade in saliva.[12] However, limited few data suggest that omega-3 PUFAs may have the potential as a host modulatory therapeutic for the prevention and/or adjuvant of periodontal disease management.[13]

Recently, visfatin was discovered as a novel adipocytokine produced mainly in the visceral adipose tissues and has insulin-like functions.[14] In human studies, visfatin was also detected in blood cells such as monocytes [15], lymphocytes [16] , macrophages [17,18] as well as dendritic cells [17]. It has a pro-inflammatory function and secreted by polymorphonuclear leukocytes (PMNs) in response to inflammatory process.[19] Visfatin stimulates the release of pro-inflammatory cytokines from monocytes [17] suggesting a possible role in the development of inflammatory diseases. Visfatin might play a role in the regulation of defense and immune functions.[19] Furthermore, visfatin molecule is over-expressed in several inflammatory disorders such as acute lung injury, rheumatoid arthritis, psoriasis and inflammatory bowel diseases.[20-23] It has a principal role in the development of chronic inflammatory foci by inhibiting PMNs apoptosis.[21] Periodontal inflammation up-regulates several pro-inflammatory cytokines which eventually lead to over-ex-

pression of visfatin in periodontal tissues.[24] It was also revealed that visfatin concentrations in serum and gingival crevicular fluid (GCF) in periodontal health and disease were quantified suggesting correlated increased visfatin concentrations in serum and GCF with severity of periodontitis.[25]

Nowadays, an increased interest in saliva as a diagnostic fluid was increased among researchers. In the field of periodontics, saliva is reported to reflect the soluble mediator composition of the periodontal tissues, GCF and saliva of subjects suffering from periodontal diseases. [26] Thus, saliva contains specific biomarkers for the unique physiopathological aspects of periodontal disease, and alteration in the concentrations of these biomarkers plays an important role in diagnostic and therapeutic approaches.[27]

Hence, in the current study, we hypothesized that dietary supplementation of omega-3 PUFAs would improve the clinical outcomes of periodontal treatment and reduce salivary inflammatory markers associated with disease. Therefore, we evaluated the impact of omega-3 PUFAs in conjunction with initial periodontal therapy (IPT) on the changes in standard periodontal measurements and salivary concentrations of both visfatin and TNF-α in patients with advanced chronic periodontitis.

MATERIALS AND METHODS

Study Population

Thirty four (34) subjects were enrolled in the study from the Periodontology Clinic at the Faculty of Dentistry, Mansoura University, Egypt, between February and August, 2016. An approval for the study was obtained from Research Ethics Committee in the Faculty of Dentistry. The inclusion criteria included good systemic health (by questionnaire), untreated advanced chronic periodontitis, age 35 to 60 years, and the presence of ≥18 teeth (excluding third molars and teeth with fixed restorations or dental implants). Advanced chronic periodontitis was defined as the presence of at least 6 teeth with a probing depth (PD) >6 mm, clinical attachment loss (CAL) ≥4 mm and evident alveolar bone loss as detected in the radiographs. Exclusion criteria included presence of any systemic disease (i.e., diabetes mellitus), cancer, radiotherapy, immunosuppressive or corticosteroid therapy, smoking, pregnant or lactating women, any antibiotic taken within the previous 2 months, current use of non-steroidal anti-inflammatory drugs, and any form of previous periodontal treatment.

After clarification of all aspects of the study, the selected patients signed an informed consent for participation in the study and each one was assigned a study number in an ascending order. Enrolled patients were subjected to initial periodontal therapy (IPT) which included full mouth thorough scaling and root planing (SRP), restoration of carious lesions and food impaction areas, extraction of hopeless teeth and giving strict oral hygiene instructions for plaque control. No antibiotics were prescribed for patients during the whole study period. After completion of IPT phase, subjects were divided randomly into two groups:

Group I (IPT): consisted 17 patients treated only with initial periodontal therapy (IPT) to act as control group.

Group II (IPT+Omega-3): included 17 patients treated with IPT followed by dietary supplementation with two grams of fish oil (Omega 3 Plus™ capsules, produced by SEDICO Pharmaceutical Co., 6 October City-Egypt) divided twice daily for three months. Each Omega 3 Plus capsule contains 1000 mg fish oil (EPA/DHA 30%) and 100mg wheat germ oil.

After taking medical and dental histories from enrolled individuals, complete thorough clinical examination was performed and a comprehensive treatment plan was scheduled. IPT was performed

for all enrolled patients and included full mouth SRP by hand and ultrasonic instruments in addition to giving oral hygiene instructions. SRP was performed by the same periodontist (SE).

At baseline (i.e. before commencement of initial periodontal therapy) and after 3 months of therapy, clinical measurements including Plaque Index (Turesky modification of Quigley-Hein, PI) [28] , Modified Gingival Index (MGI) [29] , bleeding on probing (BOP) scores (dichotomous), probing pocket depth (PD) and clinical attachment level (CAL) were measured. Subjects came to the clinic every month during the course of the study (3 months) to replenish their medication. Remaining medications were quantified as a measure of compliance. At each evaluation visit (monthly), all patients were evaluated for plaque control and examined for detection of the presence of any oral soft and hard tissue abnormalities. Moreover, patients in group II were asked about any adverse reactions due to medication intake.

Saliva sampling and analysis

At baseline and 3 months, whole unstimulated saliva samples (5 ml) samples were collected by asking the patients to expectorate into polypropylene tubes before measuring the clinical parameters. The saliva samples were centrifuged to remove debris and immediately frozen at –70°C until all samples were collected. Salivary visfatin and TNF-α levels were quantified by ELISA. The human visfatin and TNF-α ELISA kits (R&D systems, Minneapolis, USA) were utilized to quantify these analytes in saliva samples according to the manufacturers' recommendations. Salivary concentrations of both visfatin and TNF-α assays were expressed as ng/ml and pg/ml concentrations, respectively.

Statistics

The study power was performed with type I error $\alpha = 0.05$, $\beta = 0.17$ and $1-\beta = 0.83$. The power was calculated as $p = 0.8332$ and the estimated minimum sample size required was 15 per each group to achieve a power of 80%. The whole study data were explored for normality by using Kolmogrov-Smirnov test. Parametric data were presented as mean ± SD except for salivary visfatin and TNF-α levels which were represented as mean ± SEM (standard error of the mean). Demographic variables and baseline data were compared by unpaired t-test. Student t test was used for the data obtained at baseline and at 3 months after therapy, to determine whether there were significant differences between treatment groups. Data were analysed with the Statistical Package for Social Science (SPSS) program, version 19. The significance limit was set at 5%.

RESULTS

The demographic features regarding age, age range and sex in test and control groups are shown in Table1. The aforementioned features exhibited no significant difference between groups. The same table also demonstrated that there were no statistically significant variation between the two groups regarding the average values (±SD) of body mass index (BMI), number of teeth, plaque index (PI), modified gingival index (MGI), bleeding on probing score (BOP), probeable pocket depth (PD) and clinical attachment loss (CAL) as well as the mean (±SEM) of salivary levels of both visfatin and TNF-α measured as ng/ml and pg/ml, respectively ($p > 0.05$). All patients returned for scheduled maintenance and follow-up visits and there were no dropouts. No abnormalities were observed in oral soft and hard tissue examinations. Patients did not report any gastrointestinal disorders or other adverse events due to intake of omega-3 capsules.

The full mouth mean values of both groups regarding periodontal measurements at baseline and at 3 months after therapy are exhibited in Table 2. The PI reduction for the IPT and omega-3 groups at 3 month showed non-significant variations.

Moreover, there was no statistically significant difference between the two groups regarding MGI and BOP after therapy (P> 0.05). However, PD and CAL in the omega-3 group were significantly improved compared to the control values at 3 months (P< 0.001). The mean values of pocket depth reduction and clinical attachment gain were calculated in control and omega-3 groups after 3 months as shown in Fig.1A and B. The mean PD reductions in the control and omega-3 groups were 1.09±0.6 mm and 2.52±0.55 mm, respectively; with statistically significant difference, p< 0.001 (Fig.1A). In the same context, the mean CAL gain in the control and omega-3 groups were 1.24±0.47mm and 2.38±0.44 mm, respectively; with statistically significant variation, p< 0.001 (Fig.1B).

The mean (±SEM) values of salivary visfatin levels of both groups at baseline and after therapy are shown in Figure 2A. The mean values of salivary visfatin levels were 33.42±2.42 ng/ml in the omega-3 group and 32.27±3.25 ng/ml in the control group at baseline (P > 0.05). After therapy, both omega-3 and control group showed a significant reduction in visfatin concentrations (reduced to 18.78±2.17 and 17.51±2.84 ng/ml, respectively) when compared to baseline values (P< 0.001). However, no significant variation of visfatin levels was noted between groups at 3- month values (P> 0.05).

Furthermore, the mean (±SEM) values of salivary TNF-α levels of both groups at baseline and after therapy are shown in Figure 2B. The mean values of salivary TNF-α levels were 12.67±1.12 pg/ml in the omega-3 group and 13.61±1.14 pg/ml in the control group at baseline (P> 0.05). Both groups demonstrated significant reduction with regard to baseline at 3 months. At 3 months, the mean value of TNF-α level was 6.72±1.17 pg/ml in the omega-3 group and 8.97±1.09 pg/ml in the control group, and these values were statistically significant compared to baseline values (P< 0.001) and to each other (P< 0.01).

TABLE (1) Demographic Features and Baseline Data of Study Groups

Variable	**IPT (n=17)**	**IPT+Omega-3 (n=17)**
Age, year (M±SD)	47.82±2.21	45.75±2.05
Age range, year (min.-max.)	41-50	40-49
Female, n (%)	9 (53)	8 (47)
BMI (Kg/m$^{2)}$	25.3±1.4	26.1±1.2
No. of teeth	25.1±2.1	24.1±3.2
PI score	2.38±0.76	2.43±0.36
MGI score	2.27±0.13	2.32±0.19
BOP score	0.88±0.09	0.91±0.25
PD (mm)	4.46±0.57	4.76±0.84
CAL (mm)	5.08±0.46	5.16±0.52
Salivary Visfatin (ng/ml) (M±SEM)	33.42±2.42	32.27±3.25
Salivary TNF-α (pg/ml) (M±SEM)	13.61±3.14	12.76±2.51

p- value is not statistically significant (Unpaired t-test; p > 0.05).

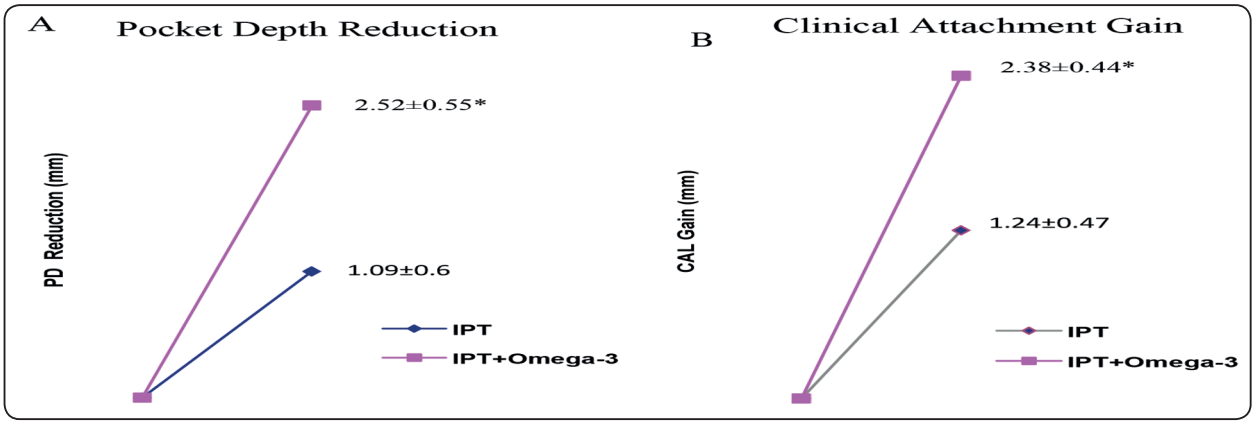

Fig. (1) Panels A and B exhibited the mean (±SD) values of full mouth pocket depth (PD) reduction and clinical attachment (CAL) gain which were significantly greater in IPT+Omega-3 group compared to IPT group after therapy; Student t test;*p<0.001.

TABLE (2) Periodontal Measurements of Study Groups at Baseline and After 3 Months of Therapy

Variable	IPT (n=17)		IPT+Omega-3 (n=17)	
	baseline	after 3 M.	baseline	after 3 M.
PI score	2.38±0.76	0.86±0.47*	2.43±0.36	0.79±0.35*
MGI score	2.27±0.13	0.72±0.14*	2.32±0.19	0.66±0.16*
BOP	0.88±0.13	0.14±0.09*	0.91±0.15	0.12±0.06*
PD (mm)	4.46±0.57	3.37±0.64*	4.76±0.84	2.24±0.27*#
CAL (mm)	5.08±0.46	3.84±0.49*	5.16±0.52	2.78±0.38*#

**** p-value is statistically significant compared to baseline values (Student t-test; p < 0.001).***

 # p-value is statistically significant compared to 3 months control values (Student t-test; p < 0.001).

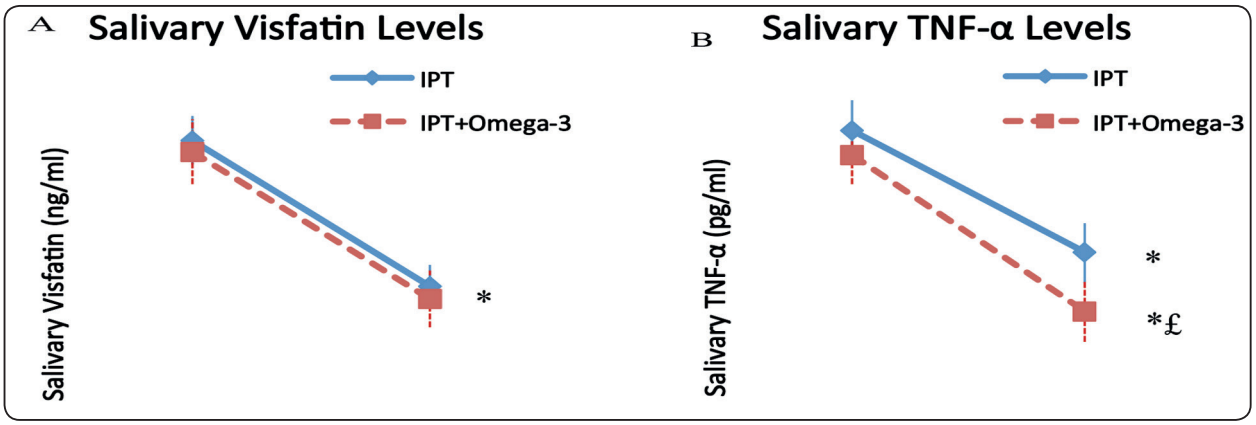

Fig. (1) Panels A and B exhibited the mean (±SD) values of full mouth pocket depth (PD) reduction and clinical attachment (CAL) gain which were significantly greater in IPT+Omega-3 group compared to IPT group after therapy; Student t test;*p<0.001.

DISCUSSION

There is a large body of evidence indicating that omega-3 PUFAs are important viable agents in controlling inflammation in several inflammatory diseases.[30-32] Nevertheless, it is not completely understood how omega-3 PUFAs affect periodontal disease outcomes.[12] The objective of our study was to determine whether the adjunctive use of omega-3 PUFAs oral supplementation in chronic periodontitis patients would have an impact on clinical parameters and salivary markers of periodontal disease. In total, our results showed that adjunctive omega-3 PUFAs supplementation with IPT significantly improved clinical and salivary biochemical outcomes in comparison to IPT alone. In our study, all the clinical parameters showed significant improvements at 3 months compared to baseline in both groups and this comes in accordance with previous investigations.[12,33,34] Similar to our study groups, the effect of dietary supplementation of omega-3 PUFAs with SRP on clinical periodontal indices in chronic periodontitis patients was investigated in two studies, which showed contrary results. The first study [35] demonstrated that dietary supplementation of omega-3 PUFAs with initial periodontal therapy showed no impact on clinical parameters while the second reported the opposite.[33] Unlike our study groups, Rosenstein and his colleagues revealed no effect of omega-3 PUFAs on gingival index and probing depth without any periodontal intervention in periodontitis patients.[36] Another study showed no improvement in the clinical parameters of individuals with experimental gingivitis.[37]

However, in the present study, we found positive effect of omega-3 PUFAs on PD reduction and CAL gain when compared to the control group. This could be explained because we have used 2 grams of fish oil per day that contain approximately 600 mg of omega-3 PUFAs, however, the aforementioned studies used less amount of omega-3 PUFAs. Although PI, GI and BOP scores showed significant reductions after therapy in both omega-3 and control groups, they were not statistically significant compared to each other. Perhaps, this occurred because the effect of omega-3 PUFAs was too subtle to detect with the current small sample size.

In the present study, we have tested salivary visfatin as a new biomarker in evaluating the response of periodontal disease to IPT alone compared to adjunctive omega-3 PUFAs with IPT. In addition, we also assessed salivary TNF-α levels because it is an established salivary marker for periodontal disease as reported in previous studies. [38,39] A limited number of studies have used visfatin as a potential periodontal marker in saliva, serum and gingival crevicular fluid (GCF) demonstrating that it could be a new reliable periodontal biomarker.[40-42] It was reported that increased visfatin expression has been associated with a variety of inflammatory diseases.[43, 44] Visfatin stimulates the release of pro-inflammatory cytokines such as TNF-α and IL-8 by peripheral mononuclear cells.[45] Thus, it could be hypothesized that there is a strong link between visfatin and the pathogenesis of periodontitis. It was indicated that visfatin concentration in serum and GCF would be a possible marker for inflammatory activity in periodontitis.[46] In the same context, our results demonstrated that salivary visfatin levels decreased significantly in patients with severe chronic periodontitis in response to both adjunctive omega-3 supplementation with IPT and IPT alone after 3 months compared to baseline values. Previous investigations have demonstrated reductions in the salivary levels of a variety of enzymes and cytokines in response to non-surgical periodontal therapy.[47,48]

TNF-α is a proinflammatory and immunoregulatory cytokine which is crucial in the pathogenesis of numerous inflammatory diseases and can be detected in saliva in both healthy periodontium and periodontal disease.[49] It was revealed that the increased TNF-α concentration observed in periodontitis correlate strongly with the severity of tissue destruction.[50] In our study, significant reductions in TNF-α levels in saliva occurred in both groups at 3 months as compared

with baseline indicating that selected salivary biomarkers reflected periodontal status and response to periodontal therapy over time in all participants. This result is consistent with Sexton et al., who evaluated salivary TNF-α levels after SRP.[51] Most importantly, in our study, salivary TNF-α levels were significantly lower in omega-3 PUFAs group compared to control group at 3 months. Accordingly, adjunctive use of omega-3 PUFAs oral supplementation for 3 months by subjects treated for chronic periodontitis could be sufficient to resolve the inflammatory state of chronic periodontitis patients. In our study, we did not evaluate the TNF-α levels in serum; however, we might hypothesize that the significant decrease in salivary TNF-α due to omega-3 PUFAs intake could be associated with the systemic reduction of serum TNF-α levels. It could be explained as increasing the intake of omega-3 PUFAs can modify the fatty-acid composition of cells and this occurs in a dose–response over an interval of weeks to months. Based on these findings, we could speculate that the significant effect of omega-3 PUFAs on TNF-α levels only at 3 months may be associated with a time–dependent cumulative effect.

In our study, we evaluated visfatin and TNF-α levels in saliva because it is readily available and easily collected without any need for certain tools or exerting more effort by clinicians. Moreover, various mediators of periodontal tissue destruction and inflammation have been detected in the whole saliva of periodontitis patients previously.[52] The unstimulated saliva contains biological materials collected from all periodontal sites and assessment of salivary markers provide an overall image of disease status other than site- specific GCF analysis. In addition, salivary biomarkers in periodontal disease have been investigated mostly in cross-sectional studies, however, the number of available longitudinal studies is limited.[38,39,53] Thus, a 6-month longitudinal study to follow chronic periodontitis patients who received SRP with/ without omega-3 PUFAs supplementation may help to understand the effects of these markers for follow-up assessment. Limited data suggest that the dietary omega-3 PUFAs could be effective in improving resistance to extracellular pathogens.[11] However, the antimicrobial activity of omega-3 PUFAs on the subgingival pathogens in naturally occurring periodontitis have not been thoroughly studied and presents an area of interest for modifying the host response to the disease.

CONCLUSIONS

1- This study suggests that daily dietary supplementation with omega-3 PUFAs could be a promising sustainable adjunctive host modulation therapeutic for periodontal disease.

2- The combination of omega-3 PUFAs to IPT can reduce salivary TNF-α levels in advanced chronic periodontitis patients more than IPT alone.

3- IPT alone or together with omega-3 PUFAs have the ability to significantly decrease salivary visfatin concentrations in severe chronic periodontitis patients.

4- Salivary visfatin might be useful for monitoring responses to periodontal therapy. However, for visfatin to be considered as a potential periodontal biomarker in the treatment of periodontal disease, further longitudinal studies with larger sample sizes are warranted.

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