Omega-3 Fatty Acids and Low-Dose Aspirin in the Treatment of Periodontitis and Metabolic Syndrome: Case Report

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Abstract

Aims: To investigate the use of ω-3 fatty acids and low-dose aspirin as adjuncts to periodontal debridement in a patient with periodontitis and metabolic syndrome.

Methods: Periodontal and systemic parameters were assessed at baseline and 6 months. Gingival crevicular fluid was analyzed for interleukin (IL)-1β, IL-6 and interferon (IFN)-γ levels by multiplex ELISA at baseline, 3 and 6 months.

Results: The treatment was effective in reducing probing depth, clinical attachment level, bleeding on probing and plaque index, and glycated hemoglobin, triglycerides IL-1β, IL-6 and IFN-γ levels over time.

Conclusion: The adjunctive use of ω-3 and low-dose aspirin to periodontal debridement might have potential benefits in the treatment of periodontitis in a patient with metabolic syndrome.

Keywords. periodontitis, diabetes, metabolic syndrome, omega-3 fatty acids, aspirin.

Introduction

Periodontitis is an inflammatory condition associated with bacterial infection that is modified by multiple host response genes in combination with lifestyle and environmental factors (Bartold and Van Dyke, 2013). Its primary features include the loss of periodontal tissue support, manifested as clinical attachment loss and radiographically assessed alveolar bone loss, the presence of periodontal pocketing and gingival bleeding (Papapanou *et al.*, 2018). Periodontitis is a highly prevalent disease and major global health problem (Petersen, 2003). The standard procedure for the treatment of periodontitis is scaling and root planing (Cobb, 2002). However, mechanical therapy alone does not always achieve the desired clinical improvements in all patients, so adjunctive therapies have been proposed (Jones *et al.*, 1994; Feres *et al.*, 2001; Wennström *et al.*, 2001; Goodson *et al.*, 2012; Teughels *et al.*, 2013; Andere *et al.*, 2017; Araujo *et al.*, 2019). Epidemiological and clinical studies support the association between periodontitis and systemic health, including diabetes (Sanz *et al.*, 2017), cardiovascular disease (Tonetti and Van Dyke, 2013)obesity (Keller *et al.*, 2015), Alzheimer's disease (Hellvard *et al.*, 2019), and systemic immunological consequences of periodontitis (Gaudilliere *et al.*, 2019).

Diabetes is an epidemic and one of the largest global health concerns of the 21st century. Four hundred and eighteen million adults currently have diabetes worldwide, and it is estimated that diabetes incidence will double by 2025 (Aschner, 2017). Cardiovascular complications of diabetes account for most of the social and financial burden of diabetes, with profound impact on global healthcare systems (Aschner, 2017). Metabolic

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syndrome (MetS) is a cluster of major cardiovascular risk factors: diabetes and hyperglycemia, abdominal obesity, dyslipidemia, and hypertension (Alberti *et al.*, 2006). MetS is believed to originate from a pro-inflammatory state, characterized by insulin resistance (Carr *et al.*, 2004). Insulin resistance is associated with increasing body mass index (BMI) and waist circumference. High levels of circulating inflammatory cytokines, such as interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α), and oxidative stress may be underlying mechanisms linking insulin resistance, obesity and chronic non-communicable diseases (Camps and García-Heredia, 2014; Rehman and Akashi, 2016; Oguntibeju, 2019).

An association between MetS and periodontitis has been suggested. This relationship is believed to be the result of systemic oxidative stress and an exacerbated inflammatory response, mainly due to the effects of dysglycemia and obesity (Lamster and Pagan, 2017). MetS increases the probability of risk for tooth loss [odds ratio (OR) 1.39], probing depth (PD) ≥ 5mm (OR 1.37), clinical attachment level (CAL) (OR 1.19), alveolar bone loss (OR 1.25), and tooth mobility (OR 1.43) (Kaye *et al.*, 2016). Although longitudinal studies are needed to establish a clear relationship between MetS and periodontitis, it can be suggested that periodontal treatment may provide benefits to the management of patients with MetS (Lamster and Pagan, 2017).

The discovery of lipid metabolites derived from the arachidonic acid (lipoxins) and from omega-3 (ω-3) polyunsaturated fatty acids (resolvins, protectins, maresins), called specialized pro-resolving mediators (SPMs), has provided new possibilities for the treatment of chronic inflammatory diseases (Serhan *et al.*, 2000; Serhan, 2014; Van Dyke *et al.*, 2015) Moreover, it has been reported that aspirin (ASA) enhances the potential of SPMs to promote the resolution of inflammation (Serhan, 1997; Serhan *et al.*, 2002). SPMs are cell surface receptor agonists with the potential to stimulate key cellular resolution events, namely limiting polymorphonuclear neutrophil infiltration and enhancing macrophage clearance of apoptotic cells (Serhan, 2014). It has been demonstrated that nutritional supplementation with marine oils enriched with ω-3 fatty acids leads to a rapid upregulation in peripheral blood SPM concentration and reprograms peripheral blood cells toward a protective phenotype (Norling *et al.*, 2017; Souza *et al.*, 2020). Pre-clinical and clinical studies (Hasturk *et al.*, 2006; González-Périz *et al.*, 2009; Gao *et al.*, 2013; Katakura *et al.*, 2014; Wales *et al.*, 2014; Herrera *et al.*, 2015; Sorokin *et al.*, 2018) showed beneficial actions of SPMs in the treatment of several chronic inflammatory diseases, including periodontitis. In a study with rabbits, it was reported that resolvin E1, and SPM derived from dietary eicosapentaenoic acid (EPA), prevented alveolar bone loss and regenerated the gingival tissue of animals with ligature-induced periodontitis (Hasturk *et al.*, 2007).

We report a case in which we used EPA and docosahexaenoic (DHA) ω-3 fatty acids and ASA as an adjunctive therapy to periodontal debridement in a patient with periodontitis and MetS with a follow-up of 6 months.

Material and Methods

A 42-year-old female patient was referred to the Dental School of São Paulo State University (Unesp), Institute of Science and Technology (São José dos Campos, São Paulo, Brazil) for periodontal treatment in 2016. Her main complaints were tooth mobility, calculus accumulation, gingival bleeding, and smile aesthetics (Figure 1). During medical screening, the patient reported having type 2 diabetes, under treatment with metformin hydrochloride (500mg/12h). The patient presented body mass index (BMI) ≥ 30kg/m^2 and

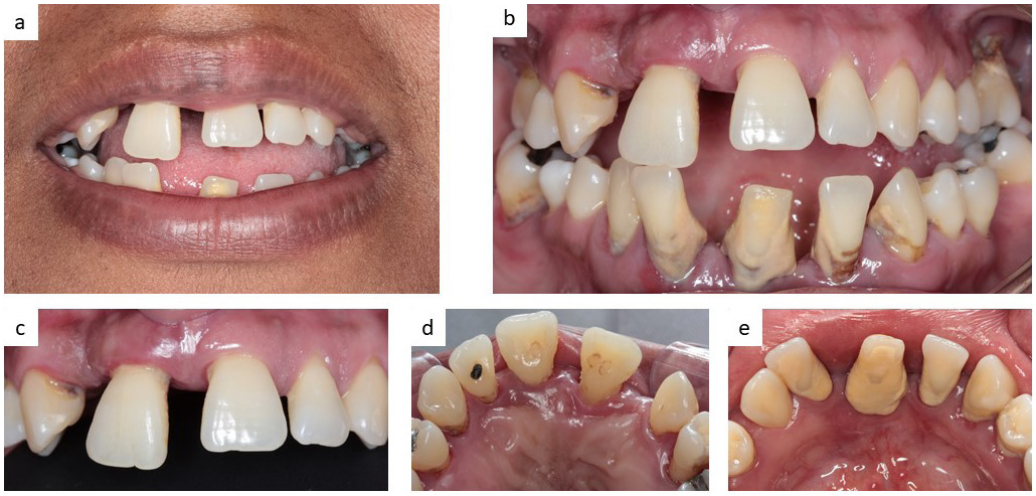

Figure 1. Baseline view of the case. (a) Smile. (b) Anterior view of the dentition. (c) Aesthetic region of the maxilla. (d) Occlusal view of the anterior region of the maxillary arch. (e) Occlusal view of anterior region of the mandible arch.

waist circumference ≥ 80cm. Blood tests revealed that hemoglobin A1c (HbA1c) level was 6.89% and triglycerides level was 223mg/dL.

A periodontal screening was performed by an experienced periodontist (CFA) and the patient was diagnosed with stage IV, grade C periodontitis (Papapanou *et al.*, 2018). Six sites per tooth were examined for pocket depth (PD), clinical attachment level (CAL), bleeding on probing (BoP), and plaque index (Pl). All teeth were examined at baseline, excluding teeth with mobility grade 3 and third molars. The treatment plan consisted of periodontal debridement followed by ω-3 fatty acids and ASA. The patient agreed with the treatment plan and signed the consent form. She reported not being allergic to fish/sea food or aspirin. The Oral Health Impact Profile (OHIP)-14 questionnaire (Slade, 1997) was applied at baseline and 6 months to assess if, or how, periodontal treatment would change the patient's perception regarding quality of life associated with oral health.

Biochemical analysis

To analyze the expression of the pro-inflammatory cytokines IL-1β, IL-6 and interferon (IFN)-γ, 2 sites with PD ≥5mm were selected and gingival crevicular fluid (GCF) was collected in a pooled sample using PerioPaper strips (Oraflow, New York, NY, United States) that were introduced in the pockets until slight resistance was felt and kept there for 30s. These were kept in a sterile microtube at -20ºC until Multiplex assay was carried out. The samples were shipped to The Forsyth Institute (Cambridge, MA, United States) under controlled temperature conditions. Multiplexed sandwich immunoassays, based on flowmetric Luminex xMAP technology, were conducted at The Forsyth Institute (Cambridge, MA, United States). Assays were carried out on a Luminex 100 Bio-plex Platform, and data were read with Bio-Plex Manager 6.1 (Bio-Rad Laboratories Inc., Hercules, United States). Biomarker analysis was performed according to manufacturer's protocols.

The first phase of the treatment consisted of basic dental therapy. Supragingival scaling was performed using an ultrasonic device (Neo Dabi Atlante, Ribeirão Preto, SP, Brazil) and periodontal curettes (Hu-Friedy, Chicago, IL, United States), followed by the extraction of hopeless teeth (numbers: 18, 27, 28, 31, 38, 42, 46), without postoperative complications. Subsequently, oral hygiene instructions were given by showing biofilm formation using crystal violet staining and instructing the patient on the use of dental floss associated with toothbrushing for daily mechanical control of supragingival biofilm.

After initial therapy, full-mouth periodontal debridement was performed in one session by an experienced periodontist (NCCS). Under local anesthesia, subgingival instrumentation using ultrasonic device (Cavitron Select, Dentsply, Yorl, PA, United States) with a subgingival tip (UI25KSF10S) (Hu-Friedy, Chicago, IL, United States) and periodontal curettes (Gracey 3/4, 5/6, 7/8, 11/12, 13/14, and mini-five 5/6) (Hu-Friedy, Chicago, IL, United States) was carried out. Each site was instrumented until root surfaces were smooth. Subgingival instrumentation was done on all roots associated with periodontal pockets, with no time limit for the procedure.

At the end of this session, the patient received 180 capsules of 1g fish oil with 540mg EPA and 360mg DHA/3 capsules (Catarinense Pharma, Joinville, SC, Brazil) and 60 tablets of 100mg Aspirin® (Bayer, São Paulo, SP, Brazil). The dosage prescribed was 3 capsules of 1g fish oil each with ω-3 fatty acids and 100mg ASA daily for 60 days. An external member of the study monitored the compliance regarding the prescribed ω-3 fatty acids and ASA by calling the patient one time/week during the supplementation/medication period. The patient received instructions to contact the clinic if she presented allergic reactions or adverse effects to the supplementation/medication. Monthly, the patient returned for supragingival biofilm and calculus removal, until the 6-month follow-up. At 3 and 6 months, periodontal screening was performed again and GCF was collected from the selected sites. At 6 months, systemic evaluations were performed, and the OHIP-14 questionnaire was reapplied.

Tooth 11 presented unfavorable prognosis at baseline, but the patient did not agree with the extraction because of its importance in the smile aesthetic. However, as the tooth continued to present mobility grade 3 after 6 months of periodontal treatment, the patient agreed with the tooth extraction. The patient received provisional partial removable dentures and was referred to the Dental Prosthesis Clinic and started supportive periodontal therapy (Figure 2).

Results

At the end of the follow-up, the patient presented significantly improved periodontal clinical parameters (p <0.01) (Table 1), reduced concentration levels of the pro-inflammatory cytokines IL-1β, IL-6, and IFN-γ (Table 2), and decreased HbA1c and triglyceride levels (Table 3). Clinical parameters and concentration levels of pro-inflammatory cytokines were assessed at baseline, 3 and 6 months after periodontal therapy. Systemic variables were assessed at baseline and 6 months after periodontal therapy. The OHIP-14 changes revealed that periodontal debridement associated with ω-3 fatty acids and ASA positively impacted the patient's perception about quality of life 6 months after therapy. At baseline, OHIP-14 was 44 and reduced to 38 after 6 months of periodontal therapy, changing from 100% of answers with the lowest scores at baseline to 79% at 6 months.

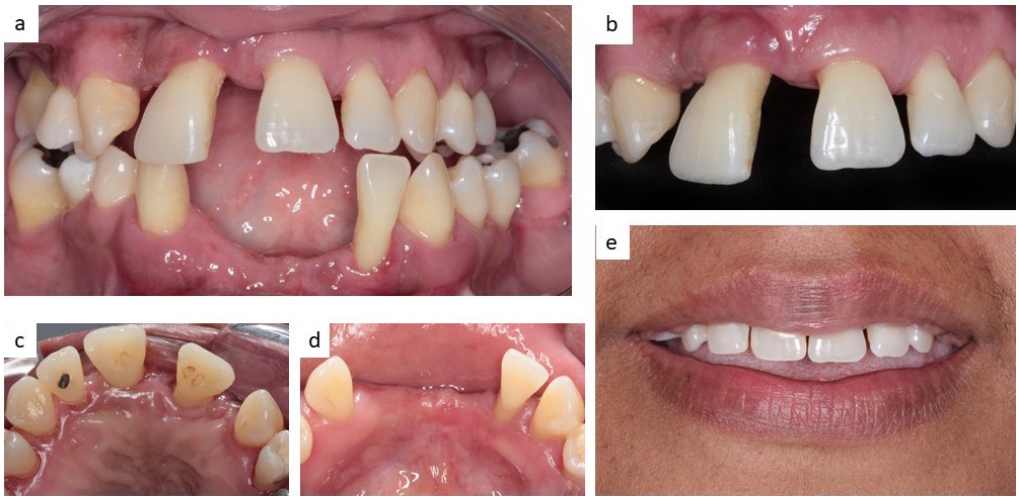

Figure 2. Clinical view of the case 6 months after periodontal therapy. (a) Anterior view of the dentition. (b) Aesthetic region of the maxilla. (c) Occlusal view of the anterior region of the maxillary arch. (d) Occlusal view of anterior region of the mandible arch. (e) After the extraction of tooth 11, the patient received provisional removable partial dentures

Table 1. Periodontal clinical parameters

Time point	Baseline	3 months	6 months	*p* value
PD (mm)	4.16	3.38*	3.08*	0.00
CAL (mm)	5.02	4.21*	3.49*	0.00
BoP (%)	72.37	40.79*	34.72*	0.00
PI (%)	69.74	40.43*	53.95*	0.00

BoP, bleeding on probing; CAL, clinical attachment level; PD, probing depth; PI, plaque index
*Statistically significant differences over time compared to baseline values. Differences in mean PD and mean CAL were assessed using T-test; p <0.01. Differences in the percentage of sites with BoP and PI were assessed using Pearson Chi-Square test, p <0.01.

Table 2. Cytokine concentration levels in gingival crevicular fluid

Time point	Baseline	3 months	6 months
IL-1β (pg/mL)	2247.00	1377.50	218.00
IL-6 (pg/mL)	203.50	142.00	131.50
IFN-γ (pg/mL)	55.00	37.00	34.50

IFN, interferon; IL, interleukin

Table 3. Systemic parameters

Time point	Baseline	6 months
HbA1c (%)	6.89	6.31
TG (mg/dL)	223.00	201.20
BMI (kg/m^2)	41.10	38.57
BP (mmHg)	130.00/80.00	126.00/77.00

BMI, body mass index; BP, blood pressure; HbA1c, glycated hemoglobin A1c; TG, triglycerides

Discussion

In this clinical case, we report the results of a patient with stage IV, grade C periodontitis, based on 2017 classification of periodontal and peri-implant diseases (Papapanou *et al.*, 2018), and MetS treated with EPA and DHA ω-3 fatty acids and ASA adjunct to periodontal debridement. This treatment protocol successfully improved periodontal clinical and immunological parameters, decrease HbA1c and triglyceride levels, and positively impacted quality of life.

Dietary supplementation with marine oil enriched with ω-3 fatty acids is an effective therapeutic approach for the treatment of inflammatory diseases. Pre-clinical studies demonstrated that nutritional interventions with ω-3 fatty acids prevented myocardial infarction (Gilbert *et al.*, 2015), aortic inflammation (Wales *et al.*, 2014), and osteoarthritis (Benabdoune *et al.*, 2016). In metabolic syndrome, supplementation with ω-3 fatty acids restrained oxidative stress through the modulation of lipoxygenase and cyclooxygenase activities (Dasilva *et al.*, 2015) and prevented renal failure, which was associated with the reduction of triglyceride levels and upregulation of SPMs (Katakura *et al.*, 2014). In a clinical study with obese women, dietary supplementation with ω-3 promoted the reduction of C-reactive protein, triglycerides and insulin resistance, which correlated with increased plasma levels of resolvins and up-regulation of the resolvin D1 receptor (Polus *et al.*, 2016). A recent clinical study investigated the relationship between ω-3 fatty acids supplementation and peripheral blood concentrations of lipid metabolites derived from ω-3 polyunsaturated fatty acids (resolvins, protectins, maresins), called SPMs. The authors demonstrated that ω-3 fatty acids supplementation leads to a time and dose-dependent increase of plasma SPM concentrations, regulating bacterial phagocytosis. Additionally, through a transcriptomic analysis of peripheral blood cells, it was observed that ω-3 reprogramed peripheral blood cell responses toward sterile and infectious stimuli (Souza *et al.*, 2020). These findings unravel mechanisms underlying the protective actions of ω-3 fatty acids and substantiate the therapeutic potential to treat inflammatory conditions.

In a placebo-controlled randomized clinical trial (RCT), periodontal clinical results of ω-3 fatty acids and ASA as an adjunctive therapy to scaling and root planing in the treatment of generalized severe periodontitis were reported (El-Sharkawy *et al.,* 2010). The authors observed mean PD and mean CAL reductions (p <0.05) and decreased salivary levels of RANKL and MMP-8 in the test group after 6 months of therapy (p <0.01). Other pilot clinical studies with shorter follow-ups evaluated the use of ω-3 fatty acids without ASA for the treatment of periodontitis, but less evident favorable results were observed (Deore *et al.*, 2014; Martinez *et al.*, 2014). Previous RCTs evaluated adjunctive therapies for nonsurgical periodontal treatment in patients with type 2 diabetes, mainly investigating systemic and local antibiotics (Botero *et al.*, 2013; Miranda *et al.*, 2014; Agarwal *et al.*, 2017), and low-level laser therapy (Macedo *et al.* 2013; Castro dos Santos *et al.*, 2016; Castro dos Santos *et al.*, 2019Springer-Verlag London. Diabetes has become a global epidemic. Its complications can have a significant impact on quality of life, longevity, and public health costs. The presence of diabetes might impair the prognosis of periodontal treatments due to its negative influence on wound healing. Antimicrobial photodynamic therapy (aPDT). A recent placebo-controlled RCT evaluated clinical and immunological actions of ω-3 and ASA as adjuncts to periodontal debridement in patients with type 2 diabetes (Castro dos Santos *et al.*, 2020). It was observed that adjunctive ω-3 and ASA after periodontal debridement provided clinical and immunological benefits to the treatment of periodontitis in patients with type 2 diabetes. In the current case report, we detected a reduction in PD, CAL, BoP, and Pl after 6 months of therapy (p <0.01). Pro-inflammatory cytokine concentration levels of IL-1β, IL-6, and IFN-γ were decreased in GCF. These results support prior reported observations suggesting that ω-3 and ASA as an adjunct to periodontal debridement can promote periodontal benefits for patients with severe periodontitis.

Hemoglobin A1c (HbA1c) is the gold-standard parameter for glycemic control and clinical management of patients with diabetes, as it represents the serum glucose levels during the 120-day life of red blood cells. Whilst chronic inflammation impairs glycemic control, high levels of HbA1c negatively affect systemic inflammation, as observed in diabetic individuals with periodontitis (Taylor, 2001). Evidence suggests that periodontal therapy decreases by 0.36% HbA1c levels, that is comparable to the effect of adjunct medication with metformin for metabolic control in patients with diabetes (Engebretson and Kocher, 2013). In this clinical case, we detected a reduction of 0.58% in HbA1c levels between baseline and 6 months.

According to the results obtained from the Multiplex assay, the concentration levels of the pro-inflammatory cytokines IL-1β, IL-6, and IFN-γ decreased over time. IL-1β and IL-6 are pro-inflammatory cytokines that have long had their roles unraveled the pathogenesis of periodontitis. In the presence of pathogens, innate host response cells recognize microbial components as "danger signals" and produce inflammatory mediators, increasing leukocyte migration and osteoclastogenesis (Garlet, 2010). Thus, a therapy that reduces concentration levels of cytokines related to innate immunity has been shown to significantly contribute to tissue homeostasis and periodontal health (Castro dos Santos *et al.*, 2020).

IL-6 is an important pro-inflammatory marker that plays a central role in host defense mechanisms against infection, inflammation, and tissue injury (Matsuda and Hirano, 1990). The chronic exposure to IL-6 leads to the impairment of insulin secretion and increases insulin resistance through the generation of oxidative stress and the potentiation of low-grade inflammation (Rehman and Akash, 2016). Thus, high plasma concentration levels of IL-6 is a risk marker for diabetes and its complications. Evidence suggests that non-surgical periodontal therapy reduces the levels of inflammatory mediators in serum (Shimada *et al.*, 2010). Therefore, reduction in GCF levels of IL-6 may positively impact systemic health and decrease the risk for diabetes complications and mortality in MetS patients.

IFN-γ is an important mediator of immunity and inflammation. It is secreted by Th1 cells and plays a key role in macrophage activation, host defense against pathogens, upregulation of IL-12, downregulation of IL-4, and mediation of Th to Treg differentiation (Hu and Ivashkiv, 2009). IFN-γ functions are mediated by direct activation of immune effector genes, including genes encoding microbicidal molecules, phagocytic receptors, chemokines, cytokines, and antigen presenting molecules (Schroder *et al.*, 2006)and appropriate regulation of macrophage function requires the integration of multiple signalling inputs derived from the recognition of host factors (e.g. interferon-γ/IFNγ. It has been observed that the use of ω-3 fatty acids and ASA as an adjunctive therapy to periodontal debridement promoted greater reduction of IFN-γ levels in GCF when compared to placebo (Castro dos Santos *et al.*, 2020). Therefore, it could be hypothesized that the immune modulation therapy with adjunctive ω-3 fatty acids and ASA promoted environmental changes in the periodontal pocket that could have altered the subgingival biofilm composition.

A previous study has shown that the use of ω-3 fatty acids and ASA in the treatment of chronic inflammatory diseases has great potential to promote the resolution of inflammation and restore homeostasis in patients with periodontitis and diabetes (Sima and Van Dyke, 2016). In this case report, we observed multiple periodontal, immunological and systemic benefits from the adjunctive use of ω-3 fatty acids EPA and DHA and ASA to periodontal debridement in a patient with periodontitis and MetS.

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Conflicts of interest

The authors declare no conflicts of interest.

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