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Evaluation of Treatment with Omega-3 Fatty Acid Supplements on Salivary Levels of Resolvin E1 in Chronic Periodontitis Patients

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Abstract

Context Under healthy conditions, inflammation proceeds through natural healing processes by an organized cycle. Similar to any other systemic infection, periodontal disease is also a manifestation of dysregulated inflammatory pathway.

Endogenous lipid mediators called resolvins and docosatrienes, produced from omega-3 fatty acid precursors, possess various immunoregulatory properties. These polyunsaturated fatty acids cannot be synthesized in body, instead these have to be taken through diet. This study aims to evaluate the effect of adjunctive treatment with daily dietary supplementation of omega-3 fatty acids in chronic periodontitis.

Aims The aim of this study was to assess periodontal parameters and salivary levels of Resolvin E1 with and without dietary supplementation of omega-3 fatty acid capsules in chronic periodontitis patients.

Methods and Materials Three-month comparative clinical study was performed on 52 patients allotted to two groups, each with 26 subjects. Both groups received an initial phase 1 therapy followed by additional dietary supplementation of 500 mg of omega-3 fatty acid capsules for 3 months for the test group. Salivary levels of Resolvin E1, periodontal parameters, including pocket probing depth (PPD), clinical attachment loss (CAL), bleeding on probing (BOP), and periodontal inflamed surface area (PISA), were assessed at baseline, 1 and 3 months after the study.

Statistical Analysis Used Data was analyzed with an unpaired t-test between the group and paired t-test for within the group comparison. *p*-Value less than 0.05 was considered significant.

Results PPD and CAL showed statistically significant differences between the two groups and within the groups

Keywords

- omega-3 Fatty acid supplements
- PUFA
- Resolvin E1
- periodontitis

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Introduction

Periodontitis is considered a multifactorial disease affecting the tooth-supporting structures, caused by specific microorganisms resulting in progressive destruction of periodontal ligament and alveolar bone with increasing probing depth formation, recession, or both.[1] An estimate of about 743 million people is shown to be affected by the disease, rendering it the sixth most common disease existing globally.[2] The primary requisite for the initiation of the disease is the presence of a bacterial biofilm. However, the influence of the host's immunological response can affect its advancement to a certain extent.

Host modulation therapy constitutes interventions through the application of local and systemic pharmaceuticals along with phase 1 therapy. Primarily the objective of such systems is to gradually slow down the rate of tissue destruction through direct regulation of various components in the inflammatory cascade. Numerous drug systems are currently under research for the same. These include nonsteroidal anti-inflammatory drugs, tetracyclines, bisphosphonates, etc.[3] Omega-3 fatty acids (PUFAs) constitute one of a kind.

Polyunsaturated fatty acids are chemical molecules with more than one carbon double bond. They comprise omega-3 (n-3), omega-6(n-6), and omega-9 (n-9) fatty acids that belong to a group of essential fatty acids that our body cannot synthesize. Thus, retrieval of these biomolecules has to be through diet alone. Acids like eicosapentaenoic acid, and docosahexaenoic acid are derived from marine products, while alpha-linolenic acid and gamma-linolenic acid are from vegetative sources.[4,5]

PUFAs synthesize specialized lipid mediators termed resolvins, which promote anti-inflammatory and pro-resolution effects elicited through previous animal models. Resolvins consist of two types of series, namely E and D, among which Resolvins E1 (RvE1) and D1 were found to minimize inflammation and postoperative pain.[6] RvE1, Resolvin D1, and Protectin D1 can obstruct the migration of neutrophils from capillaries, as well as limit the capability of neutrophil infiltration at inflammatory sites.[7–9] These compounds possess an inhibitory action on interleukin-1 beta and tumor necrosis factor-alpha.[10–12]

Improvements in probing depth as well as gingival index scores were attained in a study conducted by Salman et al, where scores were significantly lower in periodontitis subjects who received omega supplelemntation.[13]

An alternative route of administration of omega supplements was carried out in the investigation by Mishra and Shergill. Omega-3 fatty acids were topically administered in gingivitis patients for 4 weeks. Results revealed a reduction in the mean bleeding index, gingival index, and gingival redness at 4 weeks when compared to the baseline within both groups.[14]

Elkhouli, in his randomized placebo-controlled study, evaluated the success rate of systemic administration of omega-3 acids in regenerative therapy of furcation defects. The experimental group received decalcified freeze-dried bone allograft (DFDBA) + omega supplementation along with low-dose aspirin. The control group received DFDBA and placebo alone. The study report illustrated a significant reduction in all clinical variables.[15]

Hence, the purpose of the present study is to explore the effects of omega-3 PUFA as an adjunctive host modulating agent with conventional periodontal therapy. The study aims to evaluate the clinical parameters and to estimate the immunoregulatory role of RvE1 levels from saliva, with or without dietary supplementation of omega supplements, as an adjunct to scaling and root planning (SRP) in the treatment of chronic periodontitis patients.

Materials and Methods

A total of 52 patients who had reported to the outpatient department of periodontics and had given their prior informed consent were included in the study. The study design was a randomized single-blind 3-month comparative clinical study. The institutional ethical committee approved the study. (Ethical approval number: ABSM/EC 70/ 2019).

Inclusion Criteria

Inclusion criteria for the selection of participants were as follows:

1. The mean age group of 18–60 years old.
2. Patients diagnosed with chronic periodontitis, with a minimum of 3 or more periodontal sites in two jaw quadrants with probing depth ranging from 4 to 6 mm and interproximal clinical attachment loss of 1 to 4 mm.
3. Minimum complement of 20 teeth.

Exclusion Criteria

The following criteria were excluded from the study:

1. Patients with aggressive periodontitis or any systemic disorders.
2. Pregnant/lactating woman.
3. Smokers or alcoholics.
4. Patients who had received any form of periodontal treatment in the past 6 months.
5. Subjects who are taking any mineral or vitamin supplements in the past 6 months.

Omega-3 PUFAs supplementation is contraindicated during antiplatelet and anticoagulant treatment because of the synergistic effect on bleeding times when administered together.[16] All systemically healthy individuals were enrolled in this study. The subjects were randomly assigned by block randomization into two groups with 26 in each group:

GROUP A—Patients who would undergo SRP along with dietary supplementation of omega-3 fatty acids.
GROUP B—Patients who undergo SRP without dietary supplementation of omega-3 fatty acids.

Group A would receive an extra dietary supplement of omega-3 fatty acids with (500 mg -iCOSA 3 capsules) once daily for 3 months.

Clinical parameters were evaluated and compared at baseline after SRP, followed by 1 month and 3 months. These include bleeding on probing (BOP), pocket probing depth, and clinical attachment loss (CAL), and PISA score (periodontal inflamed surface area).

PISA score estimates the active inflammatory status of the periodontium. First, CAL or PPD values at six sites per tooth will be measured and utilized for the calculation of PESA (periodontal epithelial surface area), as BOP indicates the current status of inflammation; it was considered for calculating the inflammatory burden posed by the tissues. PISA is calculated by multiplying the BOP-positive sites and PESA values. PISA can be calculated with the help of an excel spreadsheet, tabulating the values of PPD as measured on six sites per tooth.[17]

Salivary levels of RvE1 were checked in saliva during baseline 1 month and after 3 months follow-up by an enzyme-linked immunosorbent assay (ELISA) kit (RvE1 ELISA kit, Make: Abbkine, Cat No: KTE62213, Det Range: 30–480 pg./mL).

Test Principle of ELISA

Human RvE1 ELISA kit employs a two-site sandwich ELISA to quantitative RvE1 in samples. An antibody specific for RvE1 has been precoated onto microplate standards and samples are pipetted into the wells and any RvE1 present is bound by the immobilized antibody. After removing any unbound substances conjugated human RvE1 detection antibody is added to the wells. Following a wash to remove any unbound horseradish peroxidase (HRP) reagent, a chromogen solution is added to the wells and color develops in proportion to the amount of RvE1 bound in the initial step. The color development is stopped and the intensity of the color is measured (►**Fig. 1**).

Statistical Analysis

Based on a 5% level of significance, 80% power, and effect size of 0.8, the samples required per group is 26, that is, a total of 52. Data will be analyzed with an unpaired t-test between the group's comparison and paired T-test for within the group comparison. A *p*-value less than 0.05 will be considered significant

Results

The mean age of the control group was 40.11 and that of the test group was 39.73. Hence, it illustrates a similar pattern of age distribution among the two groups. The total of nonvegetarians comprised was 29 and vegetarians were 23. The nonvegetarians included in the group did not report consuming fish or fish products on a daily basis (►**Tables 1** and **2**).

Out of all clinical parameters, both pocket probing depth (PPD) and CAL showed statistically significant differences both between the groups and within the groups after 3 months. With respect to these parameters, the supplementation group showed a statistical significance. However, BOP, PISA score, and resolvin levels were not statistically significant.

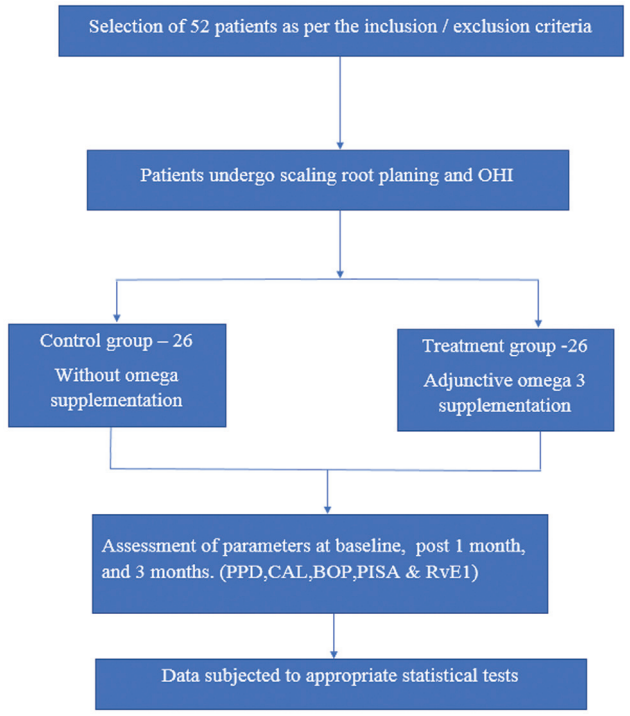

Fig. 1 Study approach. BOP, bleeding on probing; CAL, clinical attachment loss; PISA, periodontal inflamed surface area; PPD, pocket probing depth; RvE1, Resolvins E1.

Table 1 Comparison of clinical parameters between the test and control groups after 3 months using independent sample *t*-test

Parameters	Control group		Test group		p-Value
	Mean	SD	Mean	SD	
PPD	2.79	0.36	2.71	0.72	0.023[a]
CAL	2.79	0.36	2.71	0.72	0.023[a]
BOP	0.34	0.15	0.42	0.21	0.06
PISA	291.4	490.3	293.6	495.5	0.98
RSVN	36.6	9.19	37.5	8.76	0.91

Abbreviations: BOP, bleeding on probing; CAL, clinical attachment loss; PISA, periodontal inflamed surface area; PPD, pocket probing depth; RSVN, resolvin; SD, standard deviation.
[a]Statistically significant.

Discussion

PISA score was devised as an attempt to create a new classification system for periodontitis. As per the formula described by HuJoel et al, an excel spreadsheet was constructed to automatically deliver the PISA score by giving inputs of other periodontal parameters, that is, CAL, recession, and BOP per tooth.[17] As periodontal disease poses to be

Table 2 Comparison of clinical parameters within the groups after 3 months using paired *t*-test

Control group					
Parameters	Baseline		After 3 months		
	Mean	SD	Mean	SD	p-Value
PPD	2.91	0.37	2.79	0.38	0.00[a]
CAL	2.91	0.37	2.79	0.36	0.00[a]
BOP	0.35	0.16	0.34	0.15	0.15
PISA	199.4	407.2	291.4	490.9	0.35
RSVN	32.4	8.25	31.3	7.94	0.19
Treatment group					
PPD	2.86	0.69	2.68	0.67	0.000[a]
CAL	2.86	0.69	2.71	0.72	0.003[a]
BOP	0.42	0.22	0.42	0.21	0.925
PISA	199.2	407.2	293.6	495.5	0.34
RSVN	36.6	9.19	37.5	8.76	0.54

Abbreviations: BOP, bleeding on probing; CAL, clinical attachment loss; PISA, periodontal inflamed surface area; PPD, pocket probing depth; RSVN, resolvin; SD, standard deviation.
[a]Statistically significant.

inflammatory, the assessment of the total inflammatory burden posed by tissues helps the clinician to arrive at a proper treatment plan, as well as determine the prognosis. Hence, PISA stands as an effective quantitative tool for the assessment of the same. PISA also benefits as it can be amenable for the calculation of retrospective data as well.[18]

On the evaluation of clinical outcomes, both PPD and CAL showed statistical differences between the groups. The treatment group elicited a better reduction in PPD and CAL compared to the control. Also, PPD and CAL improved significantly within the test group with supplementation compared to control over 3 months. Similar results of PPD and CAL improvements were noted in the study by Kujur et al.[19] Studies conducted by El Sharkawy et al and Elkhouli also depicted a similar degree of clinical improvements in pocket depth and clinical attachment gain.[15,20] However, these studies have combined the adjunctive use of supplementation along with a low dose of aspirin that might have influenced the results.[15,20]

Omega-3 fatty acids act as metabolic substrates for neutrophil production of resolvins and protectins. They impart a protective effect on periodontitis by reducing the exaggerated inflammatory response against asaccharolytic microbial pathogens like *Porphyromonas gingivalis*. This decreased inflammatory response results in less tissue breakdown, rendering the pathogens unable to sustain their energy source.[21] Hence, the observed improvements could have been also attributed to the host modulating phenomenon of omega-3 PUFAs in patients with chronic periodontitis.

However, BOP and PISA values were not significant between the groups and within the group as well. The values of BOP and PISA were insignificant irrespective of the supplementation given. The reason for this is not clear, but one possible explanation could be that participants lose interest in oral hygiene maintenance toward the end of the observation period. Although oral hygiene reinforcement was strictly implemented, the presence of supragingival plaque was reported in a certain number of patients during final follow-ups. This predisposes to mild grade gingivitis. PISA scores were shown to positively correlate with increased plaque in literature.[22] Patients' daily dietary intake of fatty acids and their body mass index may have also affected our results.

RvE1 was also not found to elicit any characteristic difference between the groups with and without supplementation. In the cross-sectional study conducted by Tobón-Arroyave et al, the authors attempted to determine the salivary levels of various pro-resolving mediators including RvE1 in healthy and periodontitis subjects. The results showed no statistical difference in the levels of RvE1 among the clinical groups.[23]

Studies hypothesize that the metabolic activity and receptor activity on PUFAs are more likely to determine the activation of lipid mediator pathways rather than the substrate itself. Hence, impaired pathway defects or other heterogeneous effects of PUFA supplement may explain the inconsistency in resolvin levels.[24] Presently studies assessing the association between resolvin mediator and periodontal disease are very scarce in the literature.

The duration of the study was taken for 3 months as the literature suggests PUFA supplementation in the long-term course may predispose to adverse effects like impaired platelet functions and undue bleeding. This was reported in the study conducted by Thorngren and Gustafson.[25]

For estimating the biomarker levels, saliva was chosen. Biomarker levels can also be assessed by other routes like gingival crevicular fluid (GCF) or human serum. Since saliva can be abundantly obtained for sample collection and the collection procedure is less technique sensitive, this method was chosen over GCF or serum. Additionally, the constituents of GCF were also found to be ultimately merged with saliva.[26] Saliva also delivers an overall pooled estimate of all periodontal sites, rather than site-specific GCF analysis.[27] Omega-3 PUFA acts through a systemic circulation rather than topical or local action, hence saliva was a better option.

SRP stands as an integral role in any successive periodontal therapy. Therefore, it still remains a gold standard for any form of periodontal treatment.[28] Even though the adjunctive effects of PUFA were appreciable here, our study failed to compare the therapeutic efficacy of PUFA with SRP or the gold standard alone in periodontal treatment. Hence, a synergistic effect in clinical improvements is plausible to a certain extent

The strengths of the study include its single-blind randomized selection and allocation, eliminating the possibility of selection bias. All clinical parameters were conducted by a calibrated examiner. The SRP was performed by a separate examiner. The study poses certain limitations. The smaller sample size of the study design could be a limitation of the study. The effect of any adjunctive interventions has to be validated through larger population groups. The incorporation

of a certain number of nonvegetarians may have hampered the significance of the results. The therapeutic efficacy of supplementation on subjects would have been better appreciated in vegetarian subjects. For future scope, large population randomized trials with longer duration follow-ups should be carried out to validate the efficacy of PUFA supplementation

Conclusion

The following observations were made in the study:

- A statistical significance was noted with respect to pocket reduction and CAL in the omega supplementation group compared to the control. Within the groups, reduction in probing depth and CAL was significantly higher in the omega supplementation group than without supplementation over 3 months.
- There was no significant difference found in the salivary levels of RvE1 between the groups with supplementation and without supplementation.

According to the study, the systemic administration of omega capsules as an adjunct to SRP can be beneficial in the treatment of mild-to-moderate periodontitis for achieving optimal results. The association of dietary PUFA with resolvin is not significant and has to be elucidated in further studies

Conflict of Interest
None declared.

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