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ARTICLE

EFFECT OF PERIODONTAL TREATMENT PLUS OMEGA-3 IN SUBJECTS WITH PERIODONTITIS: A RANDOMIZED CONTROLLED CLINICAL TRIAL

Efecto del tratamiento periodontal más omega-3 en sujetos con periodontitis: un ensayo clínico controlado aleatorizado

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ABSTRACT

Aim: To evaluate the effect of systemic omega-3 fatty acid (Ω-3) supplementation as an adjunct to non-surgical periodontal therapy (NSPT) on the clinical and microbiological parameters of patients with periodontitis.

Material and Methods: Eighteen volunteers received NSPT combined with placebo or Ω-3 (2 g/day) for 90 days (n=10 control, n=8 intervention). Clinical parameters, including probing pocket depth (PPD), clinical attachment level (CAL), O'Leary index (OI), Bleeding on Probing (BoP) and suppuration, were recorded at baseline, and at 1, 3, and 6 months post-therapy. Microbiological evaluation at baseline, 3, and 6 months assessed the presence of *Porphyromonas gingivalis, Tannerella forsythia, Treponema denticola*, and *Fusobacterium nucleatum* using conventional polymerase chain reaction.

Results: Fifteen patients completed the study (n=9 control, n=6 intervention). No significant differences were found in demographic, clinical, or microbiological variables between groups at baseline ($p>0.05$). Both groups showed clinical improvements over time; however, no significant intergroup differences were detected in PPD, CAL, PPD reduction, CAL gain, or the percentage of sites with BoP and biofilm ($p>0.05$). Similarly, no differences were observed in the mean or changes (Δ) of shallow (PPD 1–3 mm), intermediate (PPD 4–6 mm), or deep sites (PPD ≥7 mm) between groups at any post-NSPT evaluation. No significant intra- or intergroup differences in periodontal pathogens were observed at any post-therapy evaluation.

Conclusions: Within the limitations of this study, adjunctive systemic Ω-3 did not provide additional clinical or microbiological benefits compared to NSPT alone. Further studies with larger samples and longer follow-up are required to clarify its role in periodontal therapy.

Keywords: *Fatty acids, omega-3; Periodontal debridement; Root planing; Periodontal index; Periodontitis; Periodontal diseases.*

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RESUMEN

Objetivo: Evaluar el efecto de la suplementación sistémica con ácidos grasos omega-3 (Ω-3) como complemento de la terapia periodontal no quirúrgica (TPNQ) sobre los parámetros clínicos y microbiológicos de pacientes con periodontitis.

Materiales y métodos: Dieciocho voluntarios recibieron TPNQ combinada con placebo o Ω-3 (2 g/día) durante 90 días (n=10 control, n=8 intervención). Se registraron parámetros clínicos, incluyendo la profundidad de sondaje (PS), el nivel de inserción clínica (NIC), el índice de O'Leary (IO), el sangrado al sondaje (SS) y la supuración, al inicio del estudio y a los 1, 3 y 6 meses posteriores al tratamiento. La evaluación microbiológica al inicio del estudio, a los 3 y 6 meses, determinó la presencia de *Porphyromonas gingivalis, Tannerella forsythia, Treponema denticola* y F*usobacterium nucleatum* mediante reacción en cadena de la polimerasa (PCR) convencional.

Resultados: Quince pacientes completaron el estudio (n=9 en el grupo control y n=6 en el grupo de intervención). No se encontraron diferencias significativas en las variables demográficas, clínicas o microbiológicas entre los grupos al inicio del estudio (p>0,05). Ambos grupos mostraron mejoría clínica con el tiempo; sin embargo, no se detectaron diferencias intergrupales significativas en la PS, el NIC, la reducción de PS, la ganancia de NIC, SS e IO (p>0,05). De igual manera, no se observaron diferencias en la media ni en los cambios (Δ) de los sitios superficiales (PS 1-3 mm), intermedios (PS 4-6 mm) o profundos (PS ≥7 mm). entre los grupos en ninguna evaluación posterior al tratamiento periodontal no quirúrgico (TPNQ). No se observaron diferencias intragrupales ni intergrupales significativas en los patógenos periodontales en ninguna evaluación posterior al tratamiento.

Conclusión: Dentro de las limitaciones de este estudio, la administración sistémica de Ω-3 como tratamiento complementario no proporcionó beneficios clínicos ni microbiológicos adicionales en comparación con el TPNQ solo. Se requieren estudios adicionales con muestras más grandes y un seguimiento más prolongado para aclarar su papel en la terapia periodontal.

Palabras clave: *Ácidos grasos omega-3, Desbridamiento periodontal; Aplanamiento de la raíz; Índice periodontal; Periodontitis; Enfermedades periodontales.*

INTRODUCTION

Periodontitis is a chronic inflammatory condition with multiple contributing factors, associated with dysbiotic dental biofilms.[1] It stands as the most common chronic inflammatory disease that is not communicable, posing a significant public health issue due to its high prevalence worldwide.[3] As it can lead to tooth loss and disability, periodontitis negatively affects chewing function and aesthetics,[3] contributing to social disparities and significantly reducing the quality of life.[4]

The primary clinical indicators of periodontitis include clinical attachment loss (CAL), alveolar bone loss, increased probing pocket depth (PPD), and bleeding on probing (BoP).[5] Periodontal debridement is commonly employed as a treatment method.[6] While Non-Surgical Periodontal Treatment (NSPT) yields positive short-term outcomes, these benefits may not be sustained in the long term, particularly in individuals predisposed to a chronic (hyper)inflammatory response to the microbiome due to genetic, systemic, or environmental factors.[3] Fur-

thermore, evidence suggests that accessing deeper periodontal pockets (>7mm) is challenging, and less predictable outcomes.[5]

Failure of NSPT stems largely from an inability to reverse biofilm dysbiosis and to control inflammation.[7] Thus, antimicrobial agents and immune modulators have been considered for the NSPT.[3] Host modulating therapy (HMT) offers the opportunity for modulating or reducing biofilm dysbiosis by treating aspects of chronic inflammatory host-response.[6] These are non-steroidal anti-inflammatory drugs (NSAIDs), tetracycline and bisphosphonates. However, they have adverse effects and therefore their use is limited.[6]

Recently omega-3 (Ω-3) polyunsaturated fatty acids (PUFA) have been used as host modulating therapy in various chronic inflammatory diseases, cardiovascular diseases and rheumatoid arthritis.[4] Ω-3 PUFA include eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA),[8] and are essential fatty acids, therefore, must be included in the diet.[6]

The mechanism of action of Ω-3 PUFA is attributed to the competition of EPA and DHA with arachidonic acid at two levels. Firstly, they integrate into cell membrane phospholipids, thereby reducing the levels of Arachidonic acid (AA)-derived eicosanoids.[9] Secondly, they compete as substrates for the Lipoxygenase (LOX) and Cyclooxygenase (COX) Pathways, leading to the production of EPA- and DHA-derived eicosanoids, such as protectins, maresins and resolvins.[6] These pro-resolution lipid mediators attenuate the inflammatory process, minimizing tissue damage during inflammation and facilitating the resolution of both acute and chronic inflammation.[10] Protectins inhibit T cell infiltration and promote their apoptosis.

Maresins, synthesized by macrophages from DHA, inhibit neutrophil recruitment and stimulate macrophages to clear apoptotic neutrophils.[6] Resolvins reduce neutrophil infiltration and enhance phagocyte clearance of apoptotic neutrophils.[8]

Current guidelines recommend a daily intake of 500 mg of EPA and DHA for people without cardiovascular disease, while those with a history of cardiovascular disorder should take 1 g of Ω-3 PUFA per day.[6] The use of Ω-3 fatty acids as an adjunct to NSPT has been extensively documented, with doses ranging from 300 mg to 3000 mg of combined EPA and DHA, administered over periods varying from 4 weeks to 6 months.[3] However, the results are not conclusive. On the other hand, there is no evidence of the action of this fatty acid on the composition of the periodontal flora. Consequently, the aim of this study was to assess the effect of the systemic administration of Ω-3 as an adjunct to NSPT on the clinical and microbiological variables of patients with generalised stage III, grade B periodontitis.

MATERIALS AND METHODS

Study Design

This was a 6-month, double-blind, randomized controlled trial with two arms and triple masking (participant, care provider, and outcomes assessor). The study protocol was approved by the Dentistry Faculty's Scientific Ethics Committee of the Andres Bello University's (UNAB) (Decision Nº 047) and was registered at *http://www.clinicaltrials.gov as NCT04389931.* It followed the Consolidated Standards of Reporting Trials (CONSORT) guidelines as well as the Helsinki Declaration for human research, as revised in 2013. The study was conducted by the principal investigator (MN) and secondary investigators (IP and PV).

Subject population

Thirty-one patients with generalised stage III, grade B periodontitis, were screened from the Diagnostic Unit of the UNAB School of Dentistry, Viña del Mar, 20 subjects (12 men and 8 women) met the inclusion and exclusion criteria (Table 1). However, 2 declined to participate and 18 subjects were finally randomized. The chosen participants were informed of the nature of the research, the potential risks and the compensation for participating in the study, and informed consent was obtained from each patient.

Sample Size Calculation

To calculate the minimum sample size necessary for the groups, the variance of PPD differences before and after the intervention were considered as fixed values and a standard deviation (SD) of 1.0 mm. The study by Deore *et al.*,[11] was used to obtain mean probing depth difference ± SD for the control (placebo) and experimental (Ω-3) group. Based on these calculations, it was defined that 7 subjects per group would be necessary to provide a level of significance of 0.05, a statistical power of 80% and an estimation error of 1 mm. Considering an attrition of approximately 15%, 10 subjects were included in each group.

Randomization

All subjects were assigned via randomization using the Epidat 4.0 program, with age-matched control and intervention groups. Two groups were formed for investigation: an experimental group that received NSPT plus Ω-3 and a control group that received NSPT plus placebo. The patients, the examiner, the operators who performed the periodontal treatment, and the statistician did not know which subject was assigned to each study group. Only the main investigator (MN) who assigned the randomization had knowledge of the group to which each patient belonged in the study and of the contents of the medication containers. He, therefore, was in charge of labelling the containers. To maintain a double-blind condition, the containers used for the Ω-3 and placebo presented the same characteristics of size and colour. The Ω-3 capsules and placebo tablets were visually identical.

Examiner calibration

As only one investigator (IP) performed the periodontal examination, an intra-examiner reliability assessment was conducted. This examiner (intra-class correlation coefficient of 0.97 and 0.96 for PPD and CAL) recorded all stipulated variables in a clinical file designed especially for this study. Microbiological sampling and the NSPT were performed by the other investigator (PV). For standardization, measurements were performed under the same conditions using the same type of instrument to reduce any associated bias. The instruments used for data collection were all the same design and brand and consisted of a Basic Examination Kit: Mirror, caries probe, tweezers and a manual periodontal probe (North Carolina Probe, Hu-Friedy® Manufacturing Inc., Chicago, IL, USA); plaque disclosing tablets (Curaprox © CURADEN AG, Switzerland) and number 40 sterile paper cones (Johnson & Johnson, Tokyo, Japan).

Periodontal examination

Before data collection, all subjects belonging to the study were asked for a panoramic radiograph. The following clinical variables were measured: PPD (distance in mm from the gingival margin to the bottom of the sulcus/pocket). Risk for disease progression (RDP) was defined at the patient level according to Lang and Tonetti.[12] Low risk was defined by presence ≤ 4 sites with PPD ≥ 5 mm,

5–8 sites with PPD ≥ 5 mm for moderate risk and ≥ 9 sites with PPD ≥ 5 mm for high risk. BoP (presence of immediate bleeding or up to 30 seconds after inserting the manual periodontal probe into the periodontal pocket, during the PPD measurement). CAL (distance in mm from the cement-enamel junction to the bottom of the sulcus/pocket). O'Leary index (presence of bacterial plaque on tooth surfaces that were related to the gingival margin). BoP, PPD and CAL were measured at six sites per tooth (mesiobuccal, buccal, distobuccal, distolingual/palatine, lingual/palatine and mesiolingual/palatine). O'Leary index (OI) was measured at four surfaces per tooth (buccal, mesial, lingual/palatine, distal). All teeth were evaluated, excluding the third molars. The PPD and CAL measurements were recorded to the nearest millimeter using a periodontal manual probe. BoP and OI were recorded as a percentage of sites that bled when probing and the percentage of dental surfaces with staining after using bacterial plaque finder tablets. Patients were clinically monitored at baseline, 1-, 3- and 6-months post NSPT, and periodontal maintenance was performed.

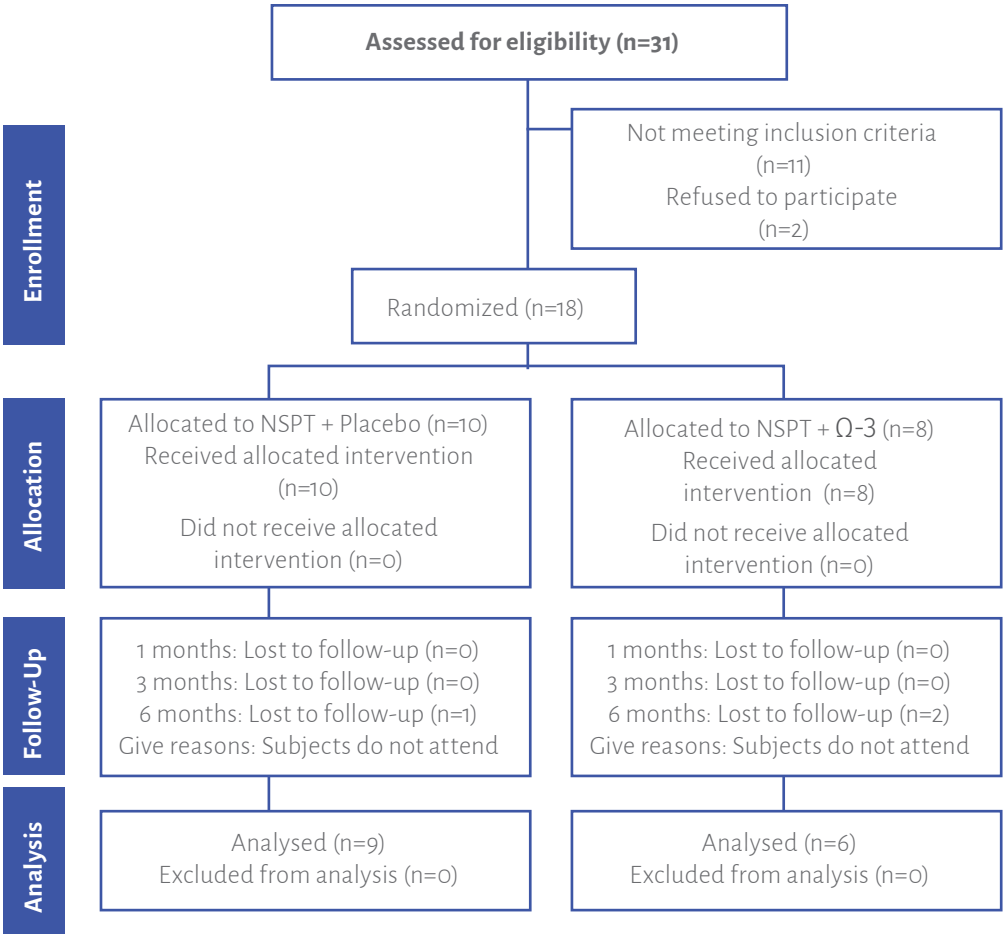
Microbiological monitoring

After the clinical parameters had been recorded, a sample of the subgingival biofilm was taken by a single trained operator (PV) from the site with the highest CAL, PPD ≥ 5 mm, BoP and/or suppuration. First, the chosen area was isolated with cotton rolls and gently air dried. Then, the supragingival deposits were carefully removed with sterile gauze. Subsequently, the samples were obtained by inserting two standardized No. 40 sterile paper cones into the deepest part of the periodontal pocket for 20 seconds to ensure the absorption of the crevicular fluid and subgingival biofilm. The microbiological variables were measured again in the third and sixth month after NSPT. Each biological sample obtained was suspended in a microfuge tube with 1 ml of distilled water and left in a 4°C container. Immediately, the samples were transported to the Laboratory of the Faculty of Life Sciences UNAB and were stored at -80°C, until the subsequent extraction of deoxyribonucleic acid (DNA). This procedure was performed following the kit manufacturer's protocol (Promega Corporation, Madison, WI, USA). The time between sampling and DNA extraction did not exceed 48 h to avoid any deterioration of the biological material. After DNA extraction, the detection of *Porphyromona gingivalis* (Pg), *Tannerella forsythia* (Tf), *Treponema denticola* (Td), and *Fusobacterium nucleatum* (Fn) was performed by amplifying a fragment of the 16S rDNA gene following the manufacturer's recommendations (Promega Corporation, Madison, WI, USA). Specific primers for each bacterium were used (see Additional file 1 Supplemental Table 1). For the reaction mixes, 0.2 ml Eppendorf microtubes were used in a 25 µL final volume protocol. Each tube contained 12.5 µL of the GoTaq® Green Master Mix (Promega Corporation, Madison, WI, USA), 0.5 µL of forward primer, 0.5 µL of reverse primer, 6 µL of nuclease-free water and 5.5 µL of DNA template.

PCR

The sample was briefly homogenized and given a quick spin to settle the contents. Then, the tubes were deposited in the thermocycler, which was programmed with a cycle of 94°C for 5 min, followed by 36 cycles of 94°C for 30 seconds, 57°C for 30 seconds and 72°C for 30 seconds, an extension cycle of 72°C for 10 min, and finally maintained at 4°C, to obtain the amplified PCR product. To observe the quality of the product, 1.5% agarose gel electrophoresis was performed for 60 min at 100 volts, and it was exposed to ultraviolet

Figure 1
Flow chart of participation in the study. Omega-3 (Ω-3), Non-Surgical Periodontal Therapy (NSPT)



light to reveal the amplified DNA products as bands on the gel. Finally, the image was captured and documented for analysis.

Periodontal intervention

Once the microbiological sample was obtained, all subjects were instructed in oral hygiene methods using a soft, straight filament toothbrush with a small head, interdental brushes and dental floss. The brushing technique used was modified Bass. Subsequently, they received one-stage full-mouth scaling and root planing (FM-SRP) performed under local anaesthesia in one or two appointments of approximately 2 h each, over a maximum period of 24 h. NSPT was performed by a single trained operator (PV), using an ultrasonic scaler (DTE®, Guilin Woodpecker Medical Instrument Co., Ltd., Guilin, Guangxi, P.R. China) and hand instruments (Gracey Curettes. Hu-Friedy® Manufacturing Inc., Chicago, IL, USA). At the end of the first treatment session, each group was provided with a sealed container containing 30 capsules, each with 1000 mg of Ω-3 PUFAs (600 mg EPA and 400 mg DHA) or 300 mg of lactose. Both products were manufactured in Galenic Pharmacy (Chile) and under the same conditions, were administered depending on the group to which the patient was assigned, intervention or control, respectively.

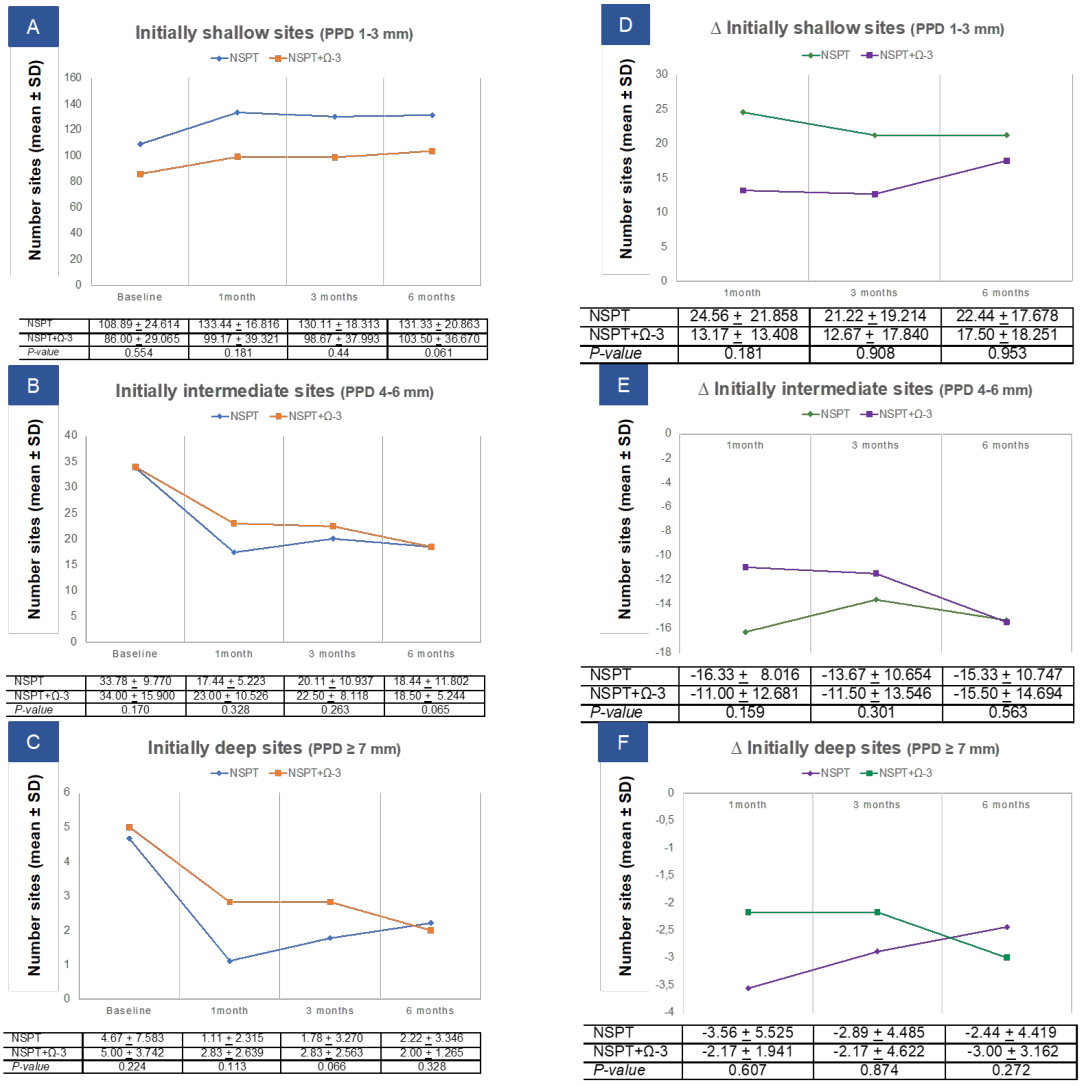
Besides, all participants were instructed to take two capsules daily for a duration of three months.

Compliance and adverse events monitoring

To monitor adherence to the treatment, patients were asked to return the empty container at the following month's visit, at which point they would receive medication for the subsequent month. This process was repeated once more, completing the three-month administration period. During this visit and at the following follow-up appointments, subjects answered a questionnaire about any self-perceived side-effects of the medication/placebo. A study investigator (IP) conducted this inquiry and was also responsible for calling the subjects every fifteen days to monitor compliance. The intervention activities carried out are summarized in a flow chart (Figure 1).

Figure 2

Mean (±SD) number of sites with different PPD (A, B, C) and Δ number sites with (D) PPD 1-3mm, (E) PPD 4-6 mm, (F) PPD ≥ 7mm, at baseline and at follow-up visits



A	Baseline	1month	3 months	6 months
NSPT	108.89 ± 24.614	133.44 ± 16.816	130.11 ± 18.313	131.33 ± 20.863
NSPT+Ω-3	86.00 ± 29.065	99.17 ± 39.321	98.67 ± 37.993	103.50 ± 36.670
P-value	0.554	0.181	0.44	0.061

B	Baseline	1month	3 months	6 months
NSPT	33.78 ± 9.770	17.44 ± 5.223	20.11 ± 10.937	18.44 ± 11.802
NSPT+Ω-3	34.00 ± 15.900	23.00 ± 10.526	22.50 ± 8.118	18.50 ± 5.244
P-value	0.170	0.328	0.263	0.065

C	Baseline	1month	3 months	6 months
NSPT	4.67 ± 7.583	1.11 ± 2.315	1.78 ± 3.270	2.22 ± 3.346
NSPT+Ω-3	5.00 ± 3.742	2.83 ± 2.639	2.83 ± 2.563	2.00 ± 1.265
P-value	0.224	0.113	0.066	0.328

D	1month	3 months	6 months
NSPT	24.56 ± 21.858	21.22 ± 19.214	22.44 ± 17.678
NSPT+Ω-3	13.17 ± 13.408	12.67 ± 17.840	17.50 ± 18.251
P-value	0.181	0.908	0.953

E	1month	3 months	6 months
NSPT	-16.33 ± 8.016	-13.67 ± 10.654	-15.33 ± 10.747
NSPT+Ω-3	-11.00 ± 12.681	-11.50 ± 13.546	-15.50 ± 14.694
P-value	0.159	0.301	0.563

F	1month	3 months	6 months
NSPT	-3.56 ± 5.525	-2.89 ± 4.485	-2.44 ± 4.419
NSPT+Ω-3	-2.17 ± 1.941	-2.17 ± 4.622	-3.00 ± 3.162
P-value	0.607	0.874	0.272

The significance of differences between groups at each time point was assessed using the unpaired *t*-test ($p>0.05$) or Mann-Whitney U test. ***:**Statistically significant difference. **PPD:** Probing Pocket Depth. **NSPT:** Non-Surgical Periodontal Therapy. **Ω-3:** Omega-3. **SD:** Standard Deviation.

Primary and secondary outcome variables

The primary outcome variable was the difference between groups of sites with PPD 1–3 mm, PPD 4–6 mm and PPD ≥ 7 mm. Secondary outcome variables were differences between groups for: RDP per patient,[12] CAL gain, BoP, OI, suppuration and presence of periodontopathogens.

Statistical Analysis

The statistical analysis was performed using IBM SPSS Statistics version 27.0 (IBM Corp., Armonk, NY, USA). A descriptive analysis was performed in which the qualitative variables were studied by frequency, while the quantitative ones were analysed by averages. For each variable, a data normality was tested using the Shapiro-Wilk test. Afterwards, an inferential analysis was carried out for the quantitative variables. The level of statistical significance was measured by the unpaired t-test and Mann-Whitney U test, while for the qualitative variables, Fisher's exact test and Chi-Square tests were performed. Significant *p*-values of <0.05 were considered, aiming to present a confidence level of 95%.

Table 1
Patient selection criteria

Inclusion criteria	Exclusion criteria
Patients with: ≥ 18 years. Classified by the American Society of Anesthesiologists (ASA) as ASA I or ASA II that were compatible with local anaesthesia procedures. At least 10 natural teeth present, excluding semi-erupted third molars. Untreated generalised stage III, grade B periodontitis, according to the AAP/EFP classification of 2018 10, with probing pocket depth (PPD) ≥ 5 mm at a minimum of four teeth and clinical attachment loss (CAL) > 4 mm, in four different quadrants.	Patients with: 1. Haemostasis disorders. 2. Taking medications associated with gingival disorders such as: anticonvulsants (phenytoin), calcium channel blockers (nifedipine), or immunosuppressive drugs 3. Any systemic diseases that affect the immunoinflammatory response. 4. Treatment with antacids on a regular basis due to chronic gastritis and/or self-medication with antacids. 5. Treatment with drugs such as: warfarin, digoxin or acetylsalicylic acid. 6. A history of allergic reactions to local anaesthetics. 7. Orthodontic appliances. 8. Antibiotic treatment in the previous 3 months. 9. History of previous periodontal treatment. 10. Pregnancy. 11. Valvular prostheses or failures of heart valves, with a risk of endocarditis. 12. Psychic and Intellectual Disability, in accordance with Chilean law number 20,584, title II, paragraph 8, article 28. 13. Consumption of more than 10 cigarettes per day. 14. Allergy to Omega 3 or seafood or its derivatives: fish, shellfish, algae, etc. 15. Lactose intolerance.

Table 2

Demographic and clinical features of patients with periodontitis. Number of patients with suppuration and mean (±SD) of the clinical parameters: PPD, CAL, of PPD reduction and CAL gain, in fullmouth at baseline and follow-up visits. Number and percentage of subjects presenting low, moderate and high. Risk for periodontitis progression at 6 months post-NSPT. Risk for disease progression according to Lang & Tonetti (2003) at 6-months post-therapies: Low (≤ 4 sites with PPD ≥ 5 mm), moderate (5-8 sites with PPD ≥ 5 mm) and high (≥ 9 sites with PPD ≥5 mm).

	Variables	Treatment groups NSPT+ Placebo (n=9)	Treatment groups NSPT+ Ω-3 (n=6)	*p*-value
Demographic features	Age (Mean ± SD)	53.89 ± 9.955	56.50 ± 8.191	0.815
	Sex (Males/Females)	6/3	5/1	0.475
Risk for disease progression	Low Risk (%)	7 (77.7)	6 (100)	0.486
	Moderate Risk (%)	2 (22.2)	0 (0.0)	
	High Risk (%)	0 (0.0)	0 (0.0)	
Time point				
Baseline	Patients with Suppuration	2/9	4/6	0.136
1 month		1/9	2/6	0.525
3 months		2/9	1/6	1.000
6 months		2/9	1/6	1.000
Baseline	PPD	3.02 ± 0.512	3.22 ± 0.651	0.456
1 month		2.43 ± 0.414	2.90 ± 0.481	0.813
3 months		2.55 ± 0.550	2.83 ± 0.488	0.602
6 months		2.43 ± 0.482	2.62 ± 0.284	0.172
6 months	PPD reduction	0.58 ± 0.220	0.50 ± 0.223	0.388
Baseline	CAL	3.63 ± 0.731	4.26 ± 1.431	0.333
1 month		3.47 ± 0.860	4.38 ± 1.699	0.241
3 months		3.61 ± 1.051	4.46 ± 1.727	0.288
6 months		3.51 ± 0.667	4.16 ± 1.335	0.278
6 months	CAL gain	0.60 ± 2.54	0.09 ± 0.747	1.000
Baseline	% of sites with BoP	63.78 ± 17.803	71.67 ± 15.293	0.391
1 month		37.22 ± 14.678	46.00 ± 5.762	0.190
3 months		35.78 ± 16.581	52.50 ± 14.977	0.069
6 months		40.22 ± 16.761	55.17 ± 12.952	0.089
Baseline	% of sites with Biofilm	78.67 ± 9.887	87.67 ± 4.926	0.061
1 month		66.78 ± 13.396	65.00 ± 11.645	0.796
3 months		72.11 ± 10.647	73.00 ± 15.582	0.897
6 months		64.78 ± 22.868	67.33 ± 16.585	0.818

The significance of differences between groups at each time point was assessed using the unpaired t-test. U de Mann-Whitney, χ^2 test and Fisher's exact test (p<0.05). **PPD:** Probing Pocket Depth. **CAL:** Clinical Attachment Level. **BoP:** Bleeding on Probing. **NSPT:** Nonsurgical Periodontal Therapy. **Ω-3:** Omega-3. **SD:** Standard Deviation.

RESULTS

This study was conducted between March and September 2016. The flow chart of the study is shown in Figure 1. Of a total of eighteen patients randomized, fifteen patients completed the study. No compliance problems were noted, and all patients followed the protocol of the study. No subjects reported any specific adverse effects. No significant differences (p>0.05) were found in any demographic, clinical or microbiological variables between treatment groups at baseline (Table 2).

There was no significant difference in the number of patients presenting with suppuration between the control and Ω-3 groups at any time point (p>0.05). In the full-mouth analysis, no statistically significant differences were observed between groups in terms of PPD, CAL, PPD reduction and CAL gain (p>0.05), or the percentage of sites with BoP and biofilm (p>0.05) at 1, 3, and 6 months post-NSPT (Table 2). Similarly, no significant differences (p>0.05) were found in the mean number of shallow, intermediate, or deep sites between treatment groups at any post-NSPT measurement point (Figures 2A, 2B, and 2C). Regarding the changes (Δ) in the number of initial shallow (PPD 1–3 mm), intermediate (PPD 4–6 mm), or deep sites (PPD ≥ 7 mm), there were also no significant differences (p>0.05) between groups at any post-NSPT time point (Figures 2D, 2E, and 2F).

With respect to RDP, all subjects in the omega-3 group were classified as "low risk" for disease progression 6 months post-treatment (Table 2).

However, no significant differences were found between the groups (p =0.48). In terms of periodontal pathogens, both inter-group and intra-group comparisons revealed no significant differences (p>0.05) at any post-NSPT measurement point (Table 3).

Table 3

Inferential analysis between the groups for the presence of periodontopathogens, according to temporality

Variables	Time point	Treatment groups		*p*-value
		NSPT+placebo (n=9)	NSPT+Ω-3 (n=6)	
Porphyromona gingivalis	Baseline	0	1	0.400
	3 months	0	0	- - -
	6 months	0	0	- - -
Tannerella forsythia	Baseline	8	5	0.756
	3 months	7	3	0.329
	6 months	4	2	1.000
Treponema denticola	Baseline	3	2	1.000
	3 months	1	2	0.525
	6 months	1	2	0.525
Fusobacterium nucleatum	Baseline	7	6	0.486
	3 months	7	6	0.486
	6 months	8	4	0.525

The significance of differences between groups at each time point was assessed using the unpaired t-test and χ^2 test (p<0.05). ***:** Statistically significant difference. **NSPT:** Non-Surgical Periodontal Therapy. **Ω-3:** Omega-3. **---:** Pg acts as a constant and it was not possible to effect a statistic analysis. The presence of this microorganism was not observed in either group in the 3rd and 6th month.

DISCUSSION

The present study evaluated the effect of the systemic administration of Ω-3 as an adjunct therapy to NSPT on the clinical and microbiological variables of patients with generalised stage III, grade B periodontitis.

At present, HMT appears to be a suitable approach for the treatment of PD. The objective of this therapy is to facilitate the swift resolution of inflammation and to promote the regeneration of periodontal tissues. This is achieved by regulating the destructive components of the host response while simultaneously upregulating the protective or regenerative responses.[13]

In terms of the study population, no significant differences in demographic or clinical characteristics were found between groups (Table 2), and therefore, a homogeneous sample was studied.

In relation to PPD, although the control group exhibited higher mean values, no significant differences were observed between the study groups at any time point for either the mean PPD or its reduction (Table 2). Similarly, previous research has also reported no differences between patients.[4,13-17] However, Maybodi *et al.*,[18] observed significant differences between study groups at 3 months[11] and Shalaby *et al.*,[19] at 6 months post NSPT.

With regard to CAL, no differences were observed between the groups (Table 2), consistent with previous studies.[4,13,14,16,17.] Conversely, four studies[11,15,18,19] reported significant differences in favour of Ω-3, while Kujur et al. observed CAL gain at the first[13] and third month post-NSPT.[11,13]

With respect to the number of shallow, intermediate, and deep sites from baseline to six months post-NSPT, both groups showed an increase in shallow sites, although no significant differences were observed between them (Figure 2A). There are no previous results available for comparison. Conversely, a reduction in the number of intermediate (Figure 2B) and deep sites (Figure 2C) was observed in both groups but were not statistically significant. These findings align with Stando *et al.*,[15] regarding intermediate pockets at 3 and 6 months but differ for deep sites at 3 months, where the Ω-3 group showed a statistically significant reduction.[15]

Regarding the delta (Δ) in the number of shallow, intermediate, and deep sites, no significant differences were observed between the study groups at any time point. This contrasts with a previous study, where the Δ baseline–3 months difference was significant in favour of the Ω-3 group.[15]

A reduction in the BoP was observed in both groups at all time points (Table 2); however, were not statistically significant. This is consistent with previous studies at 3 and 6 months,[16,18,19] but contrasts with other findings at 1,[11] 3 (4, 11, 14, 15, 17) and 6 months following NSPT (14), where a greater reduction was reported in Ω-3 group.

For OI, a reduction in the Ω-3 group was observed compared to the control group at 1, 3, and 6 months (Table 2); however, this difference was not statistically significant, which is consistent with previous research.[11,13, 14,16,17,19] In contrast, other studies have reported significant differences favouring the control group at 3 months[4] and Ω-3 group at 6 months.[15]

Regarding existing NSPT protocols, the present study was based on FM-SRP alongside an omega-3 prescription (2000 mg/24 h for 3 months), administered after the first treatment session.

Previous studies examining Ω-3 and NSPT do not specify the SRP modality used. However, when comparing FM-SRP to quadrant-based periodontal debridement, FM-SRP has demonstrated superior clinical and

microbiological outcomes,[19] which contrasts with the findings reported by Sanz *et al.*[2]

As for the dosage required to treat periodontitis, no specific recommendations have been established. The European Food Safety Authority (EFSA) recommends a daily intake of 250–500 mg of EPA and/or DHA for healthy adults.[20] In studies where Ω-3 has shown therapeutic and preventive effects on multiple chronic conditions such as cardiovascular diseases[21] and type II diabetes,[6] daily doses ranging from 2 to 6 g of Ω-3 have been employed. In relation to Periodontal Disease (PD), studies showing significant improvements in the omega-3-treated group used a dose of approximately 2000 mg/day of EPA and/or DHA.[20] Therefore, this dietary supplementation appears to be sufficient to improve periodontal parameters associated with inflammation.

The use of Ω-3 fatty acids may have beneficial effects on PD; however, the outcomes depend on both the duration of supplementation and the dosage administered.[21] The supplementation time of Ω-3 studies vary considerably; the shortest time reported was 2 months,[21] and the longest was 6 months, with different doses employed.[21]Similarly, follow-up periods also vary from 3[21] to 12 months,[20] with different Ω-3 dosages being used. A recent study,[15] demonstrated that SRP combined with adjunctive supplementation of higher doses of Ω-3 (2.6 g EPA and 1.8 g DHA) for 6 months, showed improved clinical and microbiological outcomes compared to SRP alone. This finding suggests that a higher intake of Ω-3 over a prolonged period can lead to superior outcomes.[4]

With regard to the adverse effects of Ω-3, the American Heart Association has suggested that a daily intake of 2–4 g of EPA and DHA combined,[20] and up to 5 g/day (by EFSA), is considered safe.[20] The literature mentions potential side effects, including "fishy taste," bad breath, nausea,[4,15] and gastrointestinal discomfort.[3] The Institute of Medicine has reported that doses of 2–15 g/day of EPA and/or DHA may prolong bleeding time; however, these doses have not been shown to cause clinical bleeding complications.[20] Furthermore, recent studies indicate that high doses of Ω-3 fatty acids do not affect platelet aggregation or coagulation.[20] In the present study, no participants reported adverse effects, which is consistent with findings from previous investigations.[11,17,18]

In this study, all subjects in the omega group exhibited a “low risk” of disease progression at six months post-therapy (Table 2). However, no significant differences were observed between the groups. Previous studies.[5,12] suggested that individuals with residual pockets, particularly those with probing depths PPD ≥ 5 mm, are at an increased risk of further attachment loss. In relation to the aforementioned, elevated serum levels of EPA and DHA have been reported to exert a preventive effect on the prevalence of periodontitis and reduce periodontal disease progression.[6] Additionally, long-term consumption of Ω-3 has been associated with improved periodontal health, as these fatty acids promote the resolution of inflammation and support tissue regeneration in patients with periodontitis.[4]

In relation to the bacterial aetiology of PD, evidence suggests that Ω-3 may enhance resistance to extracellular pathogens.[16] However, the antimicrobial effects of Ω-3 on subgingival microflora in periodontitis have not been extensively explored, presenting a promising area for further research into modulating the host response to the disease. The inter-group comparison at 1, 3, and 6 months post-NSPT showed no significant differences in the presence of pathogens (Table 3). This contrasts with a previous study, which reported a significant reduction in the detec-

tion frequency of Pg, Td, and Tf in the omega group at six months post-NSPT.[15]

The contents of "suppurating pockets" have been found to be polymicrobial, and predominant bacteria include Pg, *Prevotella intermedia*, Fn, and Tf.[22] However, the presence of *Porphyromonas endodontalis* and *Cutibacterium acnes* has been recently reported.[22] In relation to suppuration, in this investigation there were no significant differences between the study groups (Table 2). There are no previous results available for comparing data.

The treatment goal is "pocket closure", defined by a PPD of ≤4 mm and absence of BoP.[2] This outcome is achieved when periodontal pathogens decrease, and the root surfaces are recolonized with a higher proportion of symbiotic species.[5] However, this shift is challenging as the subgingival biofilm protects pathogens, allowing their survival in oxygen-rich areas such as the tongue, oral mucosa, saliva, and shallow pockets.[23]

While NSPT is effective, it does not always induce the ecological changes necessary for sustained clinical improvements, particularly in deep pockets with microbial invasion at the epithelial level.[5] In such cases, HMT may be indicated as an adjunct to NSPT.

Moreover, a recent investigation indicated that the aetiology of periodontitis is closely related to the bacterial dysbiosis.[24] through the overgrowth of proinflammatory Gram-negatives species.[5] Thus, periodontitis-associated microbiota can be described as inflammophilic[24]: it not only endures the inflammatory environment but also thrives in and actively sustains it.[5] On the other hand, Ω-3 may act on periodontal diseases by reducing the severity or shorten the duration of dysbiosis.[7]

The limitations of this study are as follows: First, the statistical power, which may have been insufficient to detect real differences between groups due to the small sample size and the patient attrition observed during follow-up. Second, the microbiological analysis, as only four periodontal pathogens were assessed using conventional PCR. Future research should include a larger sample size, incorporate additional pathogens associated with the aetiology of periodontitis and the presence of suppuration, and employ quantitative PCR for greater sensitivity. Moreover, extending the follow-up period to at least 12 months is recommended, considering the cumulative effect of Ω-3 supplementation reported in previous studies.

The use of Ω-3 as an adjunct to NSPT does not seem to offer any additional clinical or microbiological benefit, in agreement with the findings of Donos et al. who reported that the benefits of Ω-3 remain inconclusive. Future multi-centre trials with adequate power are required to further elucidate the role of dietary Ω-3 supplementation.[25] Based on a review,[25] the clinical practice guideline of the European Federation of Periodontology on the treatment of stage I–III periodontitis recommended “not to use Ω-3 as an adjunct to subgingival instrumentation”.[2]

CONCLUSIONS

We report the use of Ω-3 as an adjunct to NSPT does not offer any additional clinical or microbiological benefits, within the limitations of this study.

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CONFLICT OF INTERESTS

Authors report no conflicts of interest in connection with this article.

ETHICS APPROVAL

This research was approved by the Dentistry Faculty's Scientific Ethics Committee of the Andres Bello University's (UNAB), (Decision Nº 047).

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AUTHORS' CONTRIBUTIONS

Mariely Navarrete: Conceptualization, Methodology, Investigation, Project administration, Resources, Funding acquisition, Supervision, Data curation, Formal analysis, Writing – original draft, Writing – review and editing.

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Patricio Vicencio: Investigation, Methodology, Clinical procedures, Microbiological analysis, Data curation, Writing – review and editing.

All authors contributed to the interpretation of the data, critically revised the manuscript, approved the final version, and agreed to be accountable for all aspects of the work.

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SUPPLEMENTARY TABLE

Supplementary Table 1.

Forward primers, reverse primers used for 16S rDNA amplifications by PCR

Periodontal Pathogens	Forward primers	Reverse primers
Porphyromona gingivalis	5´TGT AGA TGA CTG AAA ACC 3´	5´ACG TCA TCC CCA CCT TCC TC 3´
Tannerella forsythia	5´TAC AGG GGA ATA AAA TGA GAT ACG 3´	5´ACG TCA TCC CCA CCT TCC TC 3´
Treponema denticola	5´ TAA TAC CGA ATG TGC TCA TTT ACA T 3´	5´TAC AAG AAG CAT TCC CTC TTC TTC TTA 3´
Fusobacterium nucleatum	5´GGA TTT ATT GGG CGT AAA GC 3´	5´GGC ATT CCT ACA AAT ATC TAC GAA 3´