Omega-3 and omega-6 fatty acids in primary Sjögren's syndrome: clinical meaning and association with inflammation

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ABSTRACT

Objective. *Lipid mediators derived from polyunsaturated fatty acids (FA), have been related to inflammation and immune response regulation. Herein we evaluated the intake and serum levels of ω-3 and ω-6 FA among patients with primary Sjögren's syndrome (pSS), and correlated with ocular/oral sicca symptoms, disease activity and a panel of chemokines/cytokines.*

Methods. *We included 108 patients and 100 controls. Dietary information was obtained from a food questionnaire of one-day reminder and processed using a nutritional software. Among the SS group, we measured serum ω-3 (α-linolenic acid [α-LN], eicosapentaenoic acid [EPA], docosahexaenoic acid [DHA]) and ω-6 (linoleic acid [LA], arachidonic acid [AA]) by gas chromatography flame ionisation. We scored the ESSDAI, ESSPRI, Schirmer-I test and NSWSF. In a subsample, we assessed the OSDI, ophthalmologic staining scores and measured CXCL8, CXCL10, CCL2, IL-22 and IL-21 in saliva, and CXCL8, CXCL10, CCL2 and CXCL9 in tears by Luminometry.*

Results. *ω-3 and ω-6 intake was lower in SS patients than controls, and did not correlate with serum levels. We found a negative correlation between α-LN and the OSDI and ESSDAI, as well as DHA and ESSDAI. In tears, AA positively correlated with CXCL9, whereas in saliva, α-LN, DHA and the ω3 sum negatively correlated with CCL2. We observed a negative correlation between the ω6 sum and IL-21.*

Conclusion. *pSS patients had deficient omega intake. Lower ocular symptoms, ESSDAI scores and salivary CCL2 correlated with higher ω-3 levels, possible suggesting a role in chronic inflammation. Further studies are warranted to deepen the knowledge of this association.*

Introduction

Primary Sjögren's syndrome (pSS) is an autoimmune disorder affecting mainly the exocrine glands. The natural history of the disease is characterised by a slowly progression of sicca symptoms and fatigue; however, a subset of patients would experience extraglandular activity (1). The etiopathogenic factors that lead to the loss of the immune balance and the infiltration of the exocrine glands are still unknown. However, epithelial cells seem to be key regulators of the local inflammatory process as well as the presence of diversity of immunoreactive molecules (chemokines, interleukins, etc) implicated in immune-cell homing and activation and proliferation (2).

Lipid mediators derived from polyunsaturated fatty acids, are a class of molecules related to inflammation and immune response regulation. In this sense, the polyunsaturated fatty acids omega-3 (ω-3 FA) and omega-6 (ω-6 FA) have been associated with several illnesses such as cancer, cardiovascular disease and autoimmune diseases and their balance at the cellular membranes can result in an anti-inflammatory or a pro-inflammatory status (3).

In this context, a study showed that the presence of resolvins (derived from ω-3 fatty acids) was associated with an anti-inflammatory response and the promotion of salivary epithelial integrity in a SS-like mouse model. Indeed, the use of aspirin triggered resolvin D1 and prevented chronic inflammation and enhanced saliva secretion (4). Moreover, from the clinical point of view, some studies assessing the improvement of

lacrimal function tests using oral supplementation of ω-3 and/or ω-6 FA among patients with Dry eye syndrome (DES) of diverse aetiologies including SS, had drawn controversial results (5-7).

Herein we assessed the intake and serum levels of ω-3 and ω-6 FA in patients with pSS, and correlated with the presence of oral and ocular symptoms as well as with disease activity. As a second aim, we also evaluated their correlation with oral and ocular gland dysfunction tests and with a panel of inflammatory chemokines and cytokines in saliva and tears. We hypothesised that a low ingest and low serum levels of ω-3 FA were associated with worst sicca symptoms and ocular/oral test outcomes, as well as with higher systemic activity and levels of some pro-inflammatory cytokines and chemokines implicated in SS pathogenesis.

Methods

This was a cross sectional study performed in a third level referral centre. We included 108 consecutive patients attending our Rheumatology clinic who fulfilled the 2016 ACR/EULAR classification criteria for SS (8). Patients were excluded if they were taking or had taken ω-3 or ω 6-FA oral supplements, during the previous 3 months, or had another connective tissue disease.

Participants were instructed to have a 12-hour fast, and refrained of drinking, smoking or chewing for at least 1 hour before the evaluation. All the evaluations, and serum, saliva and tears samples, were performed and collected during the morning, in a room without air conditioning or heating. All patients had a face-to-face interview with a Rheumatologist who performed a complete physical examination, evaluated disease activity using the ESSDAI score (9), measured the non-stimulated salivary flow (NSWSF) and performed the Schirmer I-test. Patients also scored the ESSPRI index for the evaluation of SS symptoms (10). We also retrospectively reviewed the patients' clinical records according to a pre-established protocol to collect serological and clinical data.

ω-3 and ω-6 food questionnaire

Dietary information regarding ω-3 and ω-6 AF consumption was obtained from a validated semi-quantitative food questionnaire of one-day reminder (11). A trained interviewer conducted the 24-h dietary recalls on each patient using standard food models. Participants reported food and beverages that were eaten on the day before the medical appointment and their respective quantities to quantify the 24-h recall. The frequency of each food item was converted into an amount of daily intake using a computerised nutritional analysis software (Food Processor Nutrition Analysis). We registered data regarding ω-3 and ω-6 AF consumption as well as the program recommendation for each of them.

For this part of the study, we also included 100 controls without any autoimmune disease matched by gender and age ± 5 years old, and followed the same procedure described above.

ω-3 and ω-6 FA serum assessment

We measured during the morning, the serum levels of ω-3 (α-linolenic acid [α-LN], eicosapentaenoic acid [EPA], docosahexaenoic acid [DHA]) and ω-6 (linoleic acid [LA], arachidonic acid [AA]).

A venous blood sample (20 mL) was collected by venipuncture of the brachial vein. Serum samples were immediately frozen at -86°C until assayed. Once defrosted, the esterified lipids from 500 μL plasma were incubated with 7 U of lipoprotein lipase (Sigma, St. Louis, MO, USA) for 1 h at 37°C. Total lipids were extracted according to Folch (12), and the fatty acids were methylated with 2 mL of methanol containing 0.002% butylated hydroxytoluene, 100 μL of toluene and 40 μL of sulfuric acid dissolved in methanol (2%). The samples were incubated at 90°C for 2 h, 1 mL of 5% NaCl was added, and then the methylated fatty acids were extracted with 2 mL of hexane (13). The organic phase was evaporated under a nitrogen stream until dryness, and the residue fatty acid methyl esters (FAME) were dissolved in 200 μL hexane for analysis by gas chromatography (Agilent 6850 GC with flame ionisation detector) using an HP-1 capillary column (30-m × 0.32- mm inner diameter with 0.25-mm film thickness; J&W Scientific, Albany, NY, USA). The injection of 1 μL of sample solution was carried out in duplicate in split mode (1:20.8) at 225°C. Helium was used as a mobile phase, with a constant flux of 0.5 mL/min, and the interface temperature was maintained at 225°C. The oven temperature was raised from 180°C to 200°C (5 min at 180°C, increased to 190°C [1°C/min]; 5 min at 190°C, increased to 200°C [1°C/min]; 10 min at 200°C). Quantification of the samples was carried out using FAME standards, and the peak areas were obtained from the generated chromatograms.

Ophthalmological evaluation

In a random subset of patients, an expert ophthalmologist in dry eye, also assessed the break-up time, van Bijsterveld and the SICCA Ocular Staining Score (OSS) scores (14). These patients also answered the Ocular Surface Disease Index (OSDI) questionnaire, a 12-item scale for the assessment of symptoms related to dry eye disease and their effect on vision (15).

Chemokine and cytokine assessment

In the subset of patients with available ophthalmological evaluation, the sterile tear strips used in their Schirmer test, as well as saliva sample gathered during the NSWSF measurement were also frozen at -86°C until assayed. Once defrosted, the tear strip samples were treated with a PBS buffer containing 0.5 M NaCl and 0.5% Tween-20. The amount of each molecule was expressed as total recovery in pg/mL as follows: first, the calculated sample in pg/mL was multiplied by the total extraction sample volume (0.2 mL) to give total pg in the extracted sample. Final pg/mL based upon Schirmer volume was calculated by dividing total pg extracted by the calculated Schirmer strip volume (pg/μL) and multiplying by 1,000. In addition, once defrosted, saliva samples were diluted in a 1:3 proportion with sterile water. We measured the levels of CXCL8, CXCL10, CCL2, IL-22 and IL-21 in saliva and

CXCL8, CXCL10, CCL2 and CXCL9 in tears by Luminometry.

This study was approved by the Institutional Biomedical Research Board of the Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán and all patients and controls gave signed informed consent to participate.

Statistical analysis

Categorical variables were compared using either χ^2 or Fisher's exact test, as appropriate; continuous variables were compared using Student's t-test and U-Mann Whitney test when non-normally distributed. We used Kruskal-Wallis test to evaluate comparison between groups. We reported non-parametric correlations using Spearman coefficients. A two-tailed $p<0.05$ will be considered significant. All analyses were performed using SPSS for Windows 22.0.

Results

We included 108 patients with pSS with both available serum sample and food intake questionnaire. The control group for the food questionnaire, was mostly women (94.6%) with a mean age of 54±13.2 years old.

Among the SS group, in nine patients, we were not able to measure at least one of the ω-FA of interest, thus we excluded them for the analysis. Furthermore, 45 out of the 99 patients also had the ophthalmological evaluation. Overall, 94.4% were women with a median age of 56.12±13.7 years, and a median disease duration of 10 years. Ninety-eight percent of the patients experienced dry eye and mouth symptoms. Table I shows the rest of the clinical and serological variables.

ω-3 and ω-6 consumption and levels

For the control group, the median intake levels of ω-3 and ω-6 were 1.91 g (0.18-4.48) and 16.78 g (1.37-25.5), respectively. In contrast, patients with SS had lower median levels of both ω-3 [0.43 g (range 0-2.7), p=1] and ω-6 [3.0 g (range 0.5-14.9), p=0.83].

Furthermore, SS patients also had low consumption of both FA according to the Food Processor recommendation, as shown in Figure 1.

Table I. Clinical and serological features of primary Sjögren's syndrome patients.

Variable	n=99
Dry eye symptoms, n (%)	97 (98)
Dry mouth symptoms, n (%)	98 (98.9)
Extraglandular feature, n (%)	78 (78.8)
Anti-Ro/SSA, n (%)	82 (82.8)
Anti-La/SSB, n (%)	51 (51.5)
Positive antinuclear antibodies, n (%)	77 (77.8)
Low C3, n (%)	9 (9.1)
Low C4, n (%)	22 (22.2)
Positive rheumatoid factor	63 (63.6)
Median ESSPRI score (range)	6.4 (5-8)
Median ESSDAI score (range)	2 (0-5)
Non-stimulated whole salivary flow mL/15 min	0.1 (0-0.5)
Median Schirmer test mm/5 min	2.0 (1-5)
Median OSDI* score (range) n=40	58.7 (39.7-73.4)
Median OSS** score (range) n=40	5.5 (3.3-7.5)
Median van Bijsterveld score (range) n=40	5.0 (3-5.6)
Median break up time (range) in seconds n=40	6.0 (6-7)

*Ocular Surface Disease Index; **SICCA Ocular Staining Score.

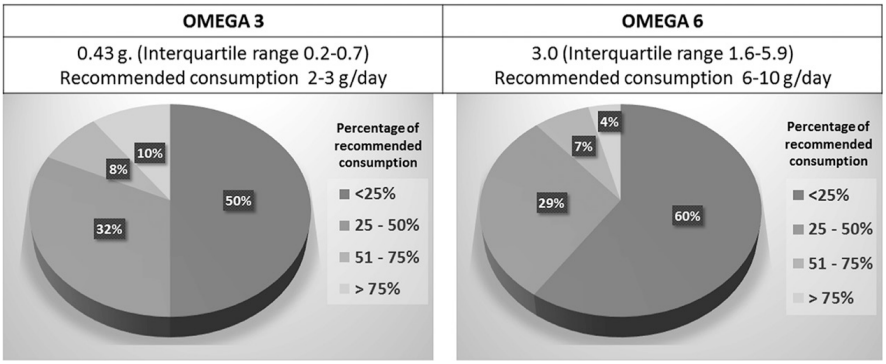

Fig. 1. ω-3 and ω-6 fatty acid consumption.

Table II shows the results of serum ω-3 and ω-6 FA among patients. We were not able to detect the presence of EPA in most of the samples. Moreover, we did not find any correlation between serum levels and food intake and each of the ω-3 and ω-6 FA (data not shown).

Correlation of serum ω-3 and ω-6 levels with clinical variables

For this analysis, we only focused on the ω-3 and ω-6 FA serum level results. We did not find any correlation with the ESSPRI, the ophthalmological variables nor with the NSWSF. On the other hand, we observed a negative correlation between α-LN (ω-3) and the OSDI score (ϱ=-0.42, p=0.01).

The ESSDAI negatively correlated with α-LN (ω-3) (ϱ=-0.22, p=0.03), DHA (ω-3) (ϱ= -0.30, p=0.01) and the ω-3 sum (α-LN + DHA) (ϱ=-0.21, p=0.05). Then we stratified the patients in three groups as follows: ESSDAI=0, ESSDAI=1-4 and ESSDAI≥ 5. We did not find differences in the evaluation of the ω-3 and ω-6 FA, except in the sum of ω-3, where the group with the highest ESSDAI score had the lowest value (Table III). We were also interested in exploring which domain of the ESSDAI might have driven our results. We focused only on the articular, hematological and biological domains that were the most prevalent in this cohort. We observed that the patients with a positive biological domain (hypocomplementaemia, high globulins and/or IgG, and positive cryoglobulins) had lower levels of α-LN (4.9 *vs*. 7.02 μg/mL, p=0.04), the sum of ω-3, (21.4 *vs*. 27.1 μg/mL p=0.03) and AA (32 *vs*. 35.3 μg/mL, p=0.04). On the other hand, those patients with a positive haematological domain (mostly leucopenia and/or lymphopenia) had

Table II. Serum levels of ω-3 and ω-6.

	Median serum levels in μg/mL (range)
α-LN*	6.6 (4.3–12.3)
DHA¶	26 (16.7–36.3)
LA¥	168.5 (89.4–287.7)
AA§	34 (24.2–50)
ω-3 sum (α-LN + DHA)	25.4 (18.4–41)
ω-6 sum (LA + AA)	205.4 (121.6–338.6)
ω-6/ ω-3 ratio	8:1
DHA/AA ratio	0.8 (0.44–1.1)

*α-linolenic acid; ¶Docosahexaenoic acid; ¥Linoleic acid; §Arachidonic acid.

Table III. Distribution of serum levels of ω-3 and ω-6 FA according to the ESSDAI score.

	ESSDAI=0 n=35	ESSDAI=1-4 n=58	ESSDAI ≥5 n=6	*p*
α-LN*, μg/mL	7 (4.7-21.4)	6.7 (4.2-11.7)	3.7 (3.2-6.2)	0.08
DHA¶, μg/mL	32.5 (21.2-36.5)	22.94 (16.2-34.1)	16.9 (12.8-26.3)	0.14
LA¥, μg/mL	184.3 (101.9-319)	166 (88.7-292.1)	102.7 (76.4-207.7)	0.26
AA§, μg/mL	35 (24.4-51.7)	34.3 (24.2-49.4)	24.8 (21-30.9)	0.22
ω-3 sum (α-LN + DHA) μg/ml	33.9 (22.6-44.7)	22.7 (14.8-35.3)	20.9 (18.6-29.7)	0.04
ω-6 sum (LA + AA) μg/ml	226.2 (121.6-370.7)	204.5 (120.3-342.3)	126.5 (99-238.6)	0.24
ω-6/ ω-3 ratio	7.4 (4-14)	8.7 (4.7-18)	4.9 (3.3-10)	0.30
DHA/AA ratio	0.87 (0.4-1.4)	0.8 (0.44-1.1)	0.67 (0.5-1.3)	0.33

*α-linolenic acid; ¶Docosahexaenoic acid; ¥Linoleic acid; §Arachidonic acid.

higher levels of LA (257.9 *vs*. 163.6 μg/mL, *p*=0.04), AA (50.5 *vs*. 32.5 μg/mL *p*=0.03), as well as the sum ω-6 (303.1 *vs*. 200.5 μg/mL *p*=0.04).

Finally, 65.7%, 63.8% and 100% of the ESSDAI=0, ESSDAI=1-4 and ESSDAI≥ 5 groups were using immunossupressors and/or prednisone. We did not find differences regarding the levels of any ω-3 and ω-6 FA among the patients with or without these treatments (data not shown).

Correlation of serum ω-3 and ω-6 levels with chemokines and cytokines

In tears, there was a positive correlation of AA and CXCL9 (ϱ=0.48, *p*=0.04). In saliva, we observed a negative correlation between α-LN (ϱ=-0.41, *p*=0.002), DHA (ϱ=-0.31, *p*=0.03) and the sum ω3 (ϱ=-0.29, *p*=0.02) with CCL2. We also observed a negative correlation between IL-21 and α-LN (ϱ=-0.28, *p*=0.03), LA (ϱ=-0.30, *p*=0.01) and the sum ω6 (ϱ=-0.29, *p*=0.01). Furthermore, there was a negative correlation with IL-22 and the ω6/ ω3 ratio (ϱ=-0.39, *p*=0.04).

Discussion

Essential ω-3 and ω-6 FA are natural modulators of inflammation activity and their balance in cellular membranes is largely dependent on dietary intake (3). Recently, the presence of resolvins, highly potent anti-inflammatory proteins derived from EPA and DHA, showed a potential link of ω-3 FA and resolution of salivary inflammation in a SS animal model (4). Moreover, in this model, the topical application of α-LN (ω-3) produced a decrease in epithelial damage, expression of inflammatory cytokines and macrophage infiltration.

Herein, we first evaluated the ω-3 and ω-6 intake through a semi-quantitative food record survey, and found a low intake of both of them in comparison to controls. In this sense, Torres-Castillo et al, evaluating Mexican healthy population with a 3-days written food record, observed an average intake of ω-3 in women of 1.3±0.8 g, a figure that is higher than our patients with pSS (16). Moreover, another study of 24 pSS patients and 22 age-matched controls, also showed a lower ω-3 intake compared to controls (17). The cause of this finding is unknown. We might argue that due to the lack of oral moisture and the presence of hypogeusia or dyspeusia, SS patients tend to change their dietary habits and avoid certain food, leading to nutritional deficiencies that might include some vitamins and omega FA acids (18).

In addition to a low ω-3 and ω-6 FA intake, we also found low serum levels; nevertheless these variables did not correlate. It is possible that the self-reported 24-h recall might not be reflecting the true omega FA intake (*i.e*. underreporting, data interpretation bias). In contrast, other types of food frequency questionnaires had showed low to moderate correlations with plasma phospholipids ω-3 levels. Moreover, studies in cardiovascular diseases described better correlations when assessing DHA and EPA, but null or low with LA and α-LN (19).

We then evaluated the correlation between the serological levels of ω-3 and ω-6 and NSWSF; and found no association. In this context, only a single pilot study of 3 month ω-3 and vitamin E supplementation vs. germ oil in SS patients failed to improve the stimulated and non-stimulated saliva production and the number of sites with gingival bleeding (markers for oral inflammation) (20). However, studies regarding the role of ω-3 FA in salivary gland SS inflammation are still needed.

In contrast, the bulk of clinical studies in ω FA had been focused in DES of diverse aetiologies (including SS), where inflammation is also a key process. For instance, at the Women's Health Study, the baseline and follow-up data showed that the highest vs. the lowest fifth of ω-3 FA intake protected against DES (21). Moreover, in a meta-analysis of 7 randomised controlled trials, the oral supplementation of ω-3 and/or ω-6 FA produced an improvement in Schirmer test and break-up time (22). Conversely, a multicentre double-blind clinical trial (n=349), of fish-derived ω-3 EPA and DHA (*vs*. placebo) for 12 months, did not show better ophthalmological staining scores, break-up time, Schirmer-I test and OSDI score (7).

Herein, in SS population, we did not find any correlation between the serum levels of ω-3 and/or ω-6 FA and any ophthalmological test. And although we did not observe a correlation between the ESSPRI, we did notice that patients with lower levels of α-LN

(ω-3) had higher scores of ocular symptoms assessed by the OSDI. On the other hand, results regarding supplementation for ocular SS sicca are also controversial. In a trial of 40 patients, the implementation of oral ω-6 (*vs*. placebo) increased PGE1 levels in tears and improved ocular signs/symptoms (23). Also, a randomised crossover trail found that a precursor of gamma-linolenic acid (ω-3) improved Schirmer test but not ocular staining, cornea sensitivity and tear lysozyme (24). In contrast, a randomised trial of gamma-linoleic acid did not show differences in ocular tests (25).

Afterwards, another finding of our study was that lower levels of α-LN, DHA and the sum of ω-3 implied higher ESSDAI scores. Similarly, Oxholm *et al*., using an arbitrary score (0-72) to assess disease activity (Grade 0=absence of glandular or extra glandular features and normal laboratory assessment, Grade 1=subclinical disease, Grade 2=mild activity, Grade 3=moderate activity, Grade 4=high activity), described that the levels of DHA negatively correlated with the clinical disease score (26). In addition, in our study, ω-3 and ω-6 FA levels did not differ with the use or not of immunosuppressors and/or steroids.

Finally, we also explored the association of ω-3 and ω-6 serum levels with some pro-inflammatory molecules involved in SS pathogenesis. For instance, Th17 cell participation has been recognised and represents a major source of IL-17A, IL-21 and IL-22 (2). IL-22 is a pronflammatory cytokine highly elevated in pSS and involved in regulating inflammation and cell proliferation. On the other hand, IL-21 participates in both inflammatory and anti-inflammatory responses, and high levels of this cytokine have been described in SS blood and tissues (27). Herein we found that IL-21 correlated with both the sum of ω-6 and α-LN (ω-3).

In addition, high concentrations of several chemokines have been reported in affected glands of patients with SS. At the present study, we focused in CCL2, CXCL8, CXCL10, and CXCL9. We found that CCL2 in saliva negatively correlated with all the ω-3 FA. This chemokine is highly expressed SS saliva and is involved in salivary gland mononuclear cell infiltration and polarisation towards a Th2 response (28). Interestingly, an in vitro study, showed that capsaicin analogues derived from EPA and DHA, reduced the production of nitric oxide, CCL20 and CCL2 (29). On the other hand, increased levels of CXCL9, CXCL10, CXCL11 and CXCR3 have been found in tears and ocular surface among patients with SS. Notwithstanding, only CXCL11 had correlated with clinical ocular tests (30). In the present work, we only observed a positive correlation among tear CXCL9 and serum levels of AA.

Finally, certain limitations need to be mentioned. First, we used gas chromatography rather than liquid chromatography with mass spectrometry. This technique is less sensible, so it is possible that it tied the detection of EPA that indeed might be low in these patients. Second, we had a low number of patients with a high ESSDAI score, so it is possible that including more active patients would have showed a stronger correlation with ω-3 serum levels. Third, the transversal design of our study did not allow us to know if serum ω-3 and ω-6 FA change through time and with disease activity.

In conclusion, pSS patients had a deficient FA omega intake. We observed lower ocular symptoms, lower ESSDAI scores and salivary levels of CCL2 among patients with higher levels of FA ω-3. Our study suggests that low serum levels of ω-3 might be implicated in the chronic inflammation status of these patients. Nevertheless, other studies are warranted to confirm our findings and to deepen the knowledge of this association.

References

1. RETAMOZO S, ACAR-DENIZLI N, RASMUSSEN A *et al*.: Systemic manifestations of primary Sjögren's syndrome out of the ESSDAI classification: prevalence and clinical relevance in a large international, multi-ethnic cohort of patients. *Clin Exp Rheumatol* 2019; 37 (Suppl. 118): S97-106.
2. HANSEN A, LIPSKY PE, DÖRNER T: Immunopathogenesis of primary Sjögren's syndrome: implications for disease management and therapy. *Curr Opin Rheumatol* 2005; 17: 558-65.
3. CALDER P: n-3 Fatty acids, inflammation and immunity: new mechanisms to explain old actions. *Proc Nutr Soc* 2013; 72: 326-36.
4. DEAN S, WANG CS, NAM K *et al*.: Aspirin Triggered Resolvin D1 reduces inflammation and restores saliva secretion in a Sjögren's syndrome mouse model. *Rheumatology* 2019; 58: 1285-92.
5. MOLINA-LEYVA I, MOLINA-LEYVA A, BUENO-CAVANILLAS A: Efficacy of nutritional supplementation with omega-3 and omega-6 fatty acids in dry eye syndrome: a systematic review of randomized clinical trials. *Acta Ophtalmol* 2017; 95: e677-85.
6. RASHID S, JIN Y, ECOIFFIER T, BARABINO S, SCHAUMBERG DA, DANA MR: Topical omega 3 and omega- 6 fatty acids for the treatment of dry eye. *Arch Ophtalmol* 2008; 126: 219-25.
7. ASBELL PA, MAGUIRE MG, PISTILLI M, YING GS, SZCZOTKA-FLYNN LB, HARDTEN DR: N-3 fatty acid supplementation for the treatment of dry eye disease. *N Engl J Med* 2018; 378: 1681-90.
8. SHIBOSKI CH, SHIBOSKI SC, SEROR R *et al*.: 2016 American College of Rheumatology/ European League Against Rheumatism classification criteria for primary Sjögren's syndrome: A consensus and data-driven methodology involving three international patient cohorts. *Ann Rheum Dis* 2017; 76: 9-16.
9. SEROR R, RAVAUD P, BOWMAN SJ *et al*.: EULAR Sjögren's syndrome disease activity index (ESSDAI): development of a consensus systemic disease activity for primary Sjögren's syndrome. *Ann Rheum Dis* 2010; 69: 1103-9.
10. SEROR R, RAVAUD P, MARIETTE X *et al*.: EULAR Sjögren's Syndrome Patient Report Index (ESSPRI): development of a consensus patient index for primary Sjögren's syndrome. *Ann Rheum Dis* 2011; 70: 968-72.
11. THOMPSON FE, BYERS T: Dietary assessment resource manual. *J Nutr* 1994; 124: 2245-317.
12. FOLCH J, LEES M, SLOANE STANLEY GH: A simple method for the isolation and purification of total lipids from animal tissues. *J Biol Chem* 1957; 226: 497-509.
13. BARAKAT HA, DOHM GL, LOESCHE P, TAPSCOTT EB, SMITH C: Lipid content and fatty acid composition of heart and muscle of the BIO 82.62 cardiomyopathy hamster. *Lipids* 1976; 11: 747-51.
14. WHITCHER JP, SHIBOSKI CH, SHIBOSKI SC *et al*.: A simplified quantitative method for assessing keratoconjunctivitis sicca from the Sjögren's Syndrome International Registry. *Am J Ophthalmol* 2010; 149: 405-15.
15. SCHIFFMAN R, CHRISTIANSON M, JACOBSEN G, HIRSHC J, REIS B: Reliability and validity of the Ocular Surface Disease Index. *Arch Ophtalmol* 2000; 118: 615-21.
16. TORRES-CASTILLO N, SILVA-GÓMEZ J, CAMPOS-PÉREZ W *et al*.: High dietary w-6: w-3 PUFA ratio is positively associated with excessive adiposity and waist circumference. *Obes Facts* 2018; 11: 344-53.
17. CERMAK JM, PAPAS AS, SULLIVAN RM *et al*.: Nutrient intake in women with primary and secondary Sjögren's syndrome. *Eur J Clin Nutr* 2003; 57: 328-34.

18. NELSON RHODUS: Qualitative nutritional intake analysis of older adults with Sjögren's Syndrome. *Gerodontology* 1988; 7: 61-9.
19. NASKA A, LAGIOU AY, LAGIOU P: Dietary assessment methods in epidemiological research: current state of the art and future prospects. *F1000Res* 2017; 6: 926.
20. SINGH M, STARK P, PALMER C, GILBARD JP, PAPAS AS: Effect of omega-3 and vitamin E supplementation on dry mouth in patients with Sjögren's syndrome. *Spec Care Dentist* 2010; 30: 225-9.
21. MILJANOVIC B, TRIVEDI K, REZA M *et al*.: Relation between dietary n-3 and n-6 fatty acids and clinically diagnosed dry eye syndrome in women. *Am J Clin Nutr* 2005; 82: 887-93.
22. LIU A, JI J: Omega-3 essential fatty acids therapy for dry eye syndrome: a meta-analysis of randomized controlled studies. *Med Sci Monit* 2014; 20: 1583-9.
23. ARAGONA P, BUCOLO C, SPINELLA R *et al*.: Systemic Omega-6 essential fatty acid treatment and PGE tear content in Sjögren's syndrome patients. *Invest Ophthalmol Vis Sci* 2005; 46: 4474-9.
24. MANTHORPE R, HAGEN PETERSEN S, PRAUSE JU: Primary Sjögren's syndrome treated with Efamol/Efavit. A double-blind cross-over investigation. *Rheumatol Int* 1984; 4: 165-7.
25. THEANDER E, HORROBIN DF, JACOBSSON LT, MANTHORPE R: Gamma linolenic acid treatment of fatigue associated with primary Sjögren's syndrome. *Scand J Rheumatol* 2002; 31: 72-9.
26. OXHOLM P, ASMUSSEN K, WIIK A, HORROBIN DF: Essential fatty acid status in cell membranes and plasma of patients with primary Sjögren's Syndrome. Correlations to clinical and immunologic variables using a new model for classification and assessment of disease manifestations. *Prostaglandins Leukot Essent Fatty Acids* 1998; 59: 239-45.
27. DI LONG D, CHEN Y, WU H, ZHAO M, LU Q: Clinical significance and immunobiology of IL-21 in autoimmunity. *J Autoimmun* 2019; 99: 1-14.
28. LEE YJ, SCOFIELD RH, HYON JY *et al*.: Salivary chemokine levels in patients with primary Sjögren's Syndrome. *Rheumatology* 2010; 49: 1747-52.
29. CIONE E, PLASTINA P, PINGITORE A *et al*.: Capsaicin analogues derived from *n*-3 polyunsaturated Fatty Acids (PUFAs) reduce inflammatory activity of macrophages and stimulate insulin secretion by β-Cells *in vitro*. *Nutrients* 2019; 11:915.
30. YOON K, PARK C, YOU I *et al*.: Expression of CXCL9,-10,-11 and CXCR3 in the tear film and ocular surface of patients with dry eye syndrome. *IOVS* 2010; 51: 643-50.