Original Article

Omega-3 fatty acids and tear cytokines modulation in dry eye patients with low omega-3 index. Have the beneficiaries been pinpointed?

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Purpose: To assess the impact of omega-3 fatty acid (O3FA) supplements on tear inflammatory cytokines in dry eye patients with an omega-3 index below 4%. **Methods**: This randomized controlled study involved 102 dry eye patients with an omega-3 index below 4%. Participants received either four capsules of O3FAs (325 mg eicosapentaenoic acid, 175 mg docosahexaenoic acid) or a placebo containing olive oil twice daily for 6 months. Patients were evaluated at baseline and 1, 3, and 6 months. The primary outcome measured changes in tear cytokines (IL-1β, IL2, IL4, IL5, IL6, IL8, IL10, IF-γ, and TNF-α). Secondary outcomes included improvements in dry eye symptoms, Nelson grade, goblet cell density, Schirmer test values, and tear film breakup time. Group means (pretreatment, 1, 3, and 6 months) were compared using repeated measure analysis of variance. **Results**: At baseline, impression cytology revealed that mRNA levels of IL-1β, IL-6, IL-8, and TNF-α were elevated by 1.6- to 2.4-fold in the O3FA group and 1.74 to 2.6-fold in the placebo group ($P = 0.123$). The O3FA group experienced a statistically significant reduction ($P < 0.05$) in tear cytokines. This group showed a 60% increase in the omega-3 index at 6 months, indicating high adherence to treatment. The dry eye symptom score, goblet cell density, and Nelson grade improved significantly in the O3FA group. However, these changes were not significant in the placebo group. **Conclusion:** This study underscores the potential advantages of O3FA supplementation in decreasing tear inflammatory cytokines in dry eye patients with an omega-3 index below 4%.

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The prevalence of dry eye disease (DED) as per the TFOS DEWS II criteria ranges from 5.4 to 44.2%.[1] A study conducted in the northern region of the subcontinent reported that 32% of young people (20–40 years) experience dry eye symptoms.[2]

Recently conducted research on dry DED patients suggests that tear hyperosmolarity initiates a cascade of signalling events in ocular surface epithelial cells, resulting in the release of inflammatory mediators and proteases. These mediators, along with hyperosmolarity, contribute to the reduction in goblet cell density and damage to the epithelial cell glycocalyx. Additionally, inflammatory mediators from activated T-cells, which are recruited to the ocular surface, further exacerbate the damage.[3]

The relationship between symptoms of dry eye and signs such as mediators of inflammation, hyperosmolarity, ocular surface staining, tear volume, TBUT, or MGD remains inconsistent. While historical literature frequently suggests that inflammation and inflammatory pathways are the primary contributors to DED, certain studies indicate that evaporative dry eye subsets display minimal increases in tear cytokines. Presumably, it cannot be universally accepted that all patients with dry eye subsets have inflammatory changes in the ocular surface.[4] Second, a significant proportion of symptomatic dry eye patients remain inadequately treated by currently available therapeutic options; it is possible that our understanding of the pathophysiology of DED may not be comprehensive.

Studies have revealed that anti-inflammatory medications like O3FAs exhibit a dichotomous effect on dry eye patients. Some patients report improvement, while others do not experience desired benefits, even with topical agents like cyclosporine and lifitegrast.[5-7]

On the contrary, oral supplementation with O3FAs seems to benefit those dry eye patients which have a low omega 3 index (<4%).[8] The effect of O3FA supplements on dry eye signs and their influence on tear inflammatory cytokines in patients with a low omega 3 index needs further evaluation. This study assessed the effects of oral omega-3 fatty acids (O3FAs) on tear cytokines in dry eye patients with a low omega-3 index. It also examined if changes in tear cytokine levels correlate with goblet cell density after omega-3 supplementation.

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Methods

A randomized, double-masked study was performed at four eye centers in northern India. The trial received approval from institutional review boards and the local ethics committee. Written informed consent was obtained from patients in accordance with the Declaration of Helsinki. The trial is registered with UMIN under the number UMIN0000649190.

Patient selection

Supervisors in regional call-centers, universities, and IT companies received a letter explaining the study's purpose and requesting participation. One university, four call centers, and two IT companies agreed to participate. Employees willing to join were invited by email to complete a questionnaire on demographics, dietary habits, symptoms, total working hours, omega-3 supplementation, and daily VDT usage over the past year. Up to three reminder emails were sent. Participants who completed the questionnaire attended a dry eye clinic for ophthalmic workups and blood tests. Symptomatic VDT users completed the "Indian Dry Eye Questionnaire," and DED was graded based on their responses [Fig. 1].[9] Some subjects meeting inclusion criteria were randomly selected to be assessed twice during one visit or the run-in period. Patients having dry eye symptoms were further advised to undergo testing for omega-3 index.

Omega-3 index

Omega-3 testing was conducted with a CAP-certified and NABL-accredited laboratory. Samples were collected from patients' homes, barcoded, packed with frozen cool packs, and delivered to the lab within 12 hours per WHO-IATA guidelines. Patients fasted for 10–12 hours and avoided alcohol 24–48 hours before testing. Whole blood was collected, and results were provided within 8–10 days.

Inclusion criteria

Symptomatic dry eye patients with an omega-3 index less than 4% were included in the study.

Exclusion criteria

Patients with current eye infections, recent refractive surgery, allergic conjunctivitis, contact lens use, herpetic eye disease, diabetes, liver or swallowing issues, aspirin or anti-coagulant therapy, or fluorescein allergy were excluded. Systemic drugs like tetracyclines and corticosteroids, and all topical medications except artificial tears, were stopped before the study. Artificial tears were prohibited 2 hours prior to testing. Most participants were enrolled in winter to reduce seasonal effects.

Dry eye questionnaire and scoring system (DESS)

Symptom	Score (maximum 18)				
	Absent (0)	Sometimes present (1)	Frequently present (2)	Always present (3)	
Itching or burning					
Sandy or gritty sensation					
Redness					
Blurring of vision					
Ocular fatigue					
Excessive blinking					

[a] Scores of 0 to 6 were mild, 6.1 to 12 were moderate, and 12.1 to 18 indicated severely symptomatic dry eye [8].

Figure 1: The Indian dry eye questionnaire. (Dry Eye Scoring System, DESS)

Sample size calculation, randomization, and masking

Sample size was calculated using the principle 'Inference for Means: Comparing means of two independent samples.' The University of British Columbia's web-based calculator was used. The sample size can also be manually calculated with the formula below.

$$N = \frac{\left[\left(Z_{\alpha/2} + Z_{\beta}\right)^2 X \{2(SD)^2\}\right]}{(\mu 1 - \mu 2)^2}$$

In this formula: N represents the sample size in each group, μ1 denotes the mean change in group 1 or the mean score at baseline, μ2 signifies the mean change in group 2 or the mean score after intervention, μ1-μ2 indicates the clinically significant difference, SD stands for standard deviation, Zα/2 is determined by the level of significance (for 5% = 1.96), and Zβ corresponds to the power (for 90% = 0.94).

A pilot study with 10 subjects measured the mean IL-6 level decrease: 2.4 in the omega-3 group and 1.6 in the placebo group, with a standard deviation of 1.2. With 90% power (alpha = 0.05) and a precision error of 5%, detecting a 20% difference required 36 subjects per group. A total of 48 participants in each group were recruited, exceeding the minimum needed for study power.

The allocation codes were generated by computer software from the department of community medicine at our institute. Stratification was done according to clinical center using a permuted-block method with randomly selected block sizes. Patients were assigned to one of two groups through parallel assignment. The codes were placed in sealed red envelopes and opened by healthcare personnel who were not involved in patient care.

In both trial groups, the regimen consisted of four soft-gel capsules taken twice daily. In the O3FA group, each capsule contained 325 mg of EPA and 175 mg of DHA, totaling 1300 mg of EPA and 700 mg of DHA per day. The placebo group received olive oil capsules comprising 70% oleic acid, 18% linoleic acid, and 12% palmitic acid. The capsules were administered twice daily for a duration of 180 days. Subjects were blinded to the capsule contents. Both types of capsules and their packaging were indistinguishable from one another. Subjects were instructed to return the bottles of study capsules at the 1-month visit, where any unused capsules were counted to assess compliance with the study protocol. Another pack containing 240 capsules was provided at this time. Participants were advised to maintain a normal diet without additional dietary supplements or increased consumption of fatty fish and to avoid over-the-counter antioxidants. Fig. 2 shows the flowchart for enrolment, randomization, intervention, follow-up, and analysis.

Outcome measures

Patients were evaluated initially and again 3 months after commencing treatment. The primary outcome assessed was the

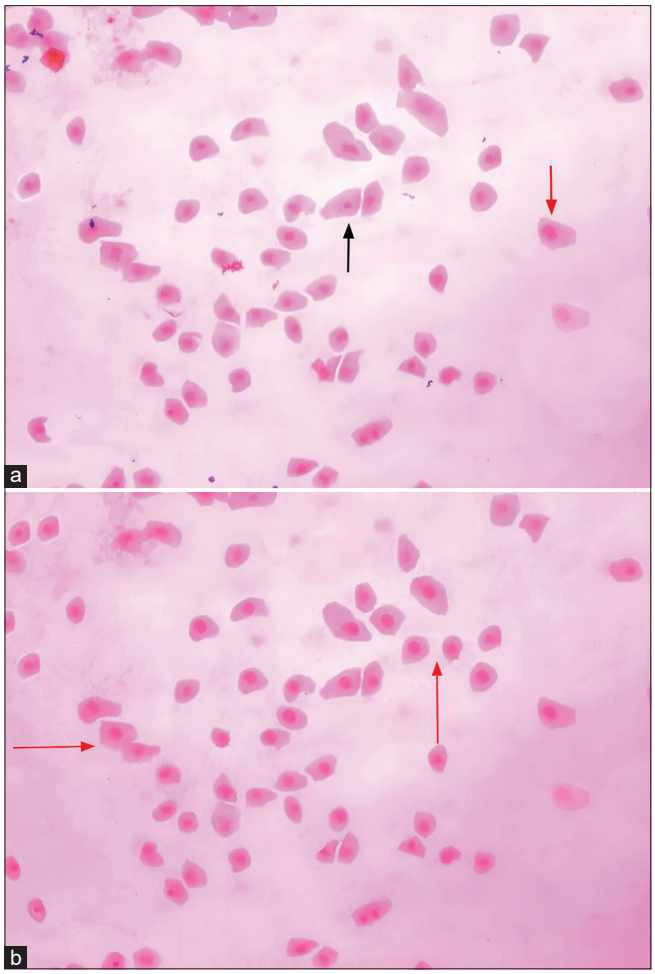


Figure 2: (a) Baseline photomicrograph of impression cytology specimen stained with periodic acid-Schiff and hematoxylin-eosin at × 400 showing squamous metaplasia (SM). Red arrow indicates a normal cell (NC), and black arrow indicates reduced nuclear–cytoplasmic ratio (SM). (b) Photomicrographs of impression cytology specimens, stained with periodic acid-Schiff and hematoxylin-eosin at × 400 magnification, showing increased nuclear-cytoplasmic at 6 months in O3FA group. Red arrows indicate increased nuclear-cytoplasmic ratio

reduction in inflammatory markers in the tears and conjunctiva of computer users over a 6-month period compared to baseline levels. The cytokines and chemokines measured included IL-1β, IL2, IL4, IL5, IL6, IL8, IL10, IF-γ, and TNF-α.

At 6 months, secondary outcomes included variations in dry eye symptom scores, tear production measured by the Schirmer test, tear film stability evaluated by TBUT, and conjunctival epithelial cell morphology and goblet cell counts.

Dry eye symptoms were evaluated on a scale of 0 to 3 for ocular fatigue, vision blurring, itching or burning, sandy or gritty sensation, and redness (DESS©). The scores were as follows: absent (0), sometimes present (1), frequently present (2), and always present (3). Dry eye severity was categorized as mild (0–6), moderate (6.1–12), and severe (12.1–18) [Fig. 1].

Ocular Examination and measurements

Participants attended the dry eye clinic between 10 AM and 12 PM. One eye per patient was randomly chosen for examination. The assessments were conducted at a temperature of 22 ± 1.4°C and a relative humidity of 48 ± 10%. An independent investigator examined the eyes, recording visual acuity and inspecting the lid margins, eyelashes, and meibomian gland openings for any blockages.

Tear collection

Nonstimulated tears were collected at the lateral canthus with 5-mL microcapillary tubes, avoiding tissue contact, at least 2 hours before dry eye exams. Twenty-microliter samples were taken between 11:00 AM and 4:00 PM within 5 minutes to minimize diurnal variation and stored at -70°C. Each was diluted tenfold; 100 µL was used to measure IL-1β, IL2, IL4, IL5, IL6, IL8, IL10, IF-γ, and TNF-α following manufacturer instructions. Tears from both eyes were pooled if one yielded insufficient volume. All assays were performed within 3 months of collection.

Tear film tests

Before tear collection, patients completed the Indian dry eye questionnaire (DESS) and underwent tear film evaluations. The investigator, unaware of the participants' treatment groups, conducted the measurements. The Tear Break-Up Time (TBUT) test was initially conducted, ensuring minimal eyelid manipulation to prevent potential interference with the results. A sterile fluorescein strip containing 1 mg of fluorescein sodium (Madhu Instruments, Delhi, India) was moistened with normal saline solution and applied to the inferior bulbar conjunctiva. Patients were instructed to blink normally, avoiding any forceful squeezing, several times to achieve even distribution of the fluorescein. The tear film was then assessed using a slit lamp equipped with a cobalt blue filter. The interval between the last complete blink and the initial appearance of a dry spot on the cornea was measured with a timer. Three consecutive measurements were obtained and averaged.

Following this procedure, the subject rested for 30 minutes. The Schirmer test with anesthesia (0.4% oxybuprocaine hydrochloride) was performed using filter paper strips measuring 5 mm by 30 mm. Each strip was folded at a right angle before use. The patient was instructed to look up while the examiner gently pulled down the lower eyelid. The bent end of the strip was then placed so it sits between the palpebral conjunctiva and bulbar conjunctiva of the eye. After the strip was positioned, the patient kept their eyes softly closed—without squeezing—for 5 minutes. The length of wetting was noted. The same examiner conducted the testing during follow-up visits.

Tear cytokine essay

Tear cytokine levels were measured using a Quansys Biosciences 9-plex assay kit for IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IFN-γ, and TNFα. Serum samples were thawed, centrifuged, and analyzed according to the manufacturer's instructions. Cytokine levels in 100 µL samples were quantified with ELISA-based chemiluminescence using Q-View Imager and QView™ Software to measure absorbance.

The detection ranges for the cytokines were as follows: IL-1β (29-218 pg/mL), IL-2 (2.13–5000 pg/mL), IL-4 (1.37–4500 pg/mL), IL-5 (1.55–4500 pg/mL), IL-6 (0.02–3000 pg/mL), IL-8 (0.16–2800 pg/mL),

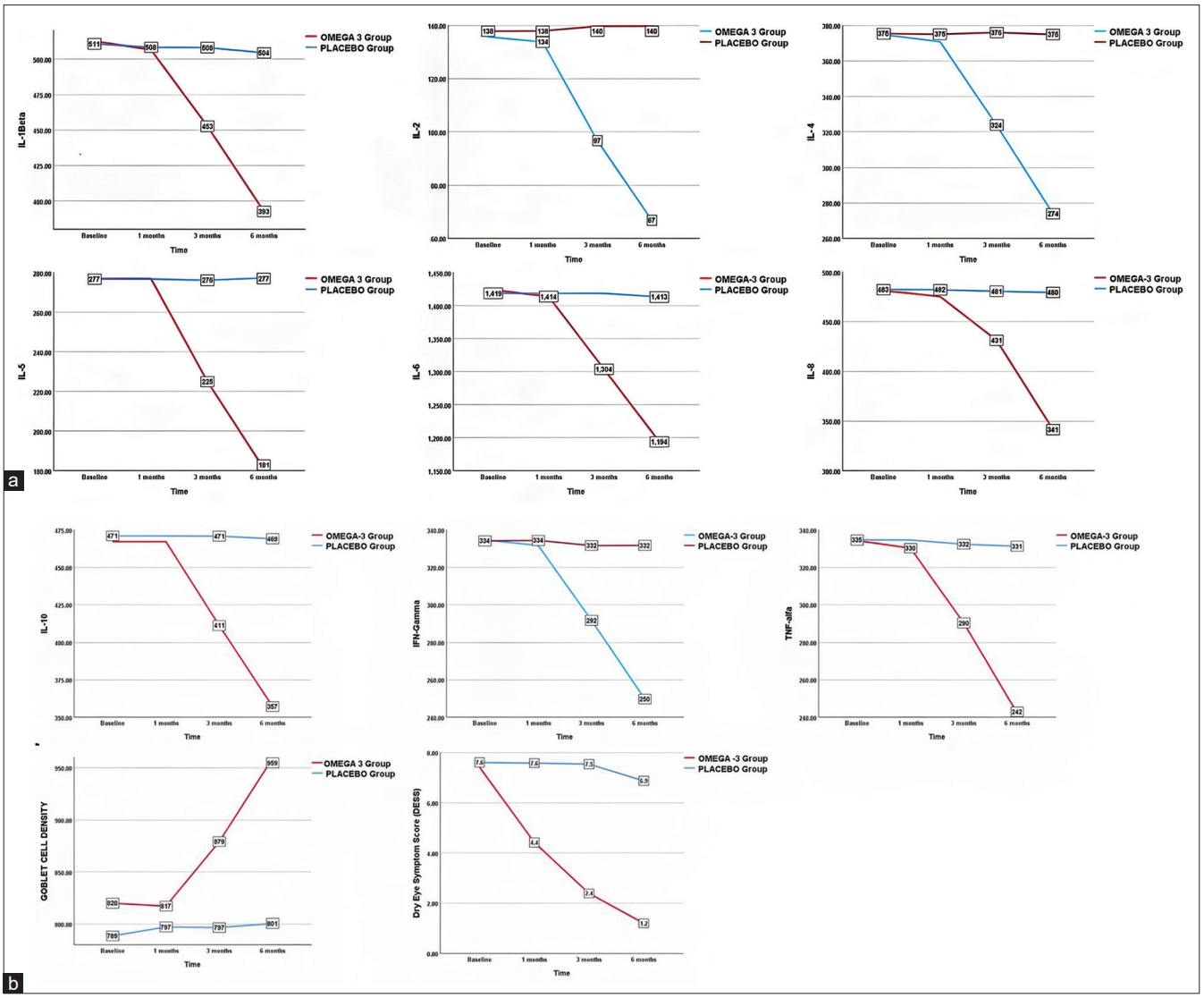


Figure 3: (a) Line diagram showing mean changes in tear cytokines in O3FA and placebo groups. (b) Line diagram showing change in tear cytokines, dry eye symptoms, and goblet cell density

IL-10 (0.25–3300 pg/mL), IF-γ (1.8-3800 pg/mL), and TNF-α (1.02–4000 pg/mL).

Conjunctival impression cytology

An independent examiner performed CIC using a standard method described previously by the authors.[10,11] Slides were viewed under light microscopes at 100X and 400X magnification to analyze cells. At least 10 high-power fields (HPFs) were examined to count goblet and epithelial cells. Goblet cell density (GCD) was calculated by dividing the goblet cells per HPF by the sampling area in square millimeters. Grades 0 and 1 were normal, while grades 2 and 3 were abnormal according to Nelson's criteria. Both computer user groups (n = 5 each) were tested for IL-1β, IL-6, IL-8, and TNF-α mRNA expression using QuantiTect SYBR Green real-time PCR to identify inflammatory cytokine sources.

Statistics

Data analysis was conducted using IBM SPSS Statistics version 30. Independent t-tests were utilized to verify baseline group similarities, while Chi-square tests were applied to proportions. Normally distributed data were reported as mean ± standard deviation (SD). Data that were not normally distributed underwent a box-cox transformation. Linear regression with a robust variance estimator was used to compare mean changes in continuous variables between groups. A one-way repeated-measures ANOVA assessed mean test value differences over 6 months of intervention (omega-3 vs. placebo). Mauchly's test indicated a violation of sphericity, $\chi 2$ (2) = 6.27, P = .043, requiring a Greenhouse–Geisser correction (ε = 0.648). A post hoc test after adjusting for multiple comparisons (Bonferroni correction) identified differences. A P value below 0.005 was considered to indicate statistical significance.

Results

Participants

The trial recruited 102 patients, with 48 in the O3FA group and 54 in the placebo group. Eight vegetarian subjects refused

to take soft-gel capsules. Eighty-four patients completed the 6-month follow-up, while 10 dropped out due to cost constraints. The study had 40 males and 44 females.

Compliance

At the 1-month follow-up, 6 patients (12.5%) in the O3FA group stopped taking supplements due to bad taste and severe gastric intolerance. In the placebo group, 7 patients (2.6%) developed mild transient rashes but did not discontinue treatment. All dropouts (n = 6) were analyzed using the last-observation-carried-forward method.

The difference in sex distribution between the two groups was not statistically significant (Chi-square test, $P = 0.564$).

Table 1 presents the mean age, mean baseline tear cytokines (IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IF-γ, and TNFα), baseline symptom score, TBUT, Nelson grade, and Schirmer score before random allocation in both groups. The intergroup differences in these variables were not statistically significant at baseline.

At baseline, impression cytology showed that mRNA levels of IL-1β, IL-6, IL-8, and TNF-α were increased by 1.6 to 2.4-fold in the O3FA group and 1.74 to 2.6-fold in the placebo group ($P = 0.123$).

At baseline, 26% of patients in the Omega-3 group had abnormal Schirmer tests, while 24% of patients in the placebo group showed the same. Abnormal TBUT was observed in 70% of the Omega-3 group and 72% in the placebo group. Additionally, 38% of the Omega-3 group had abnormal conjunctival impression cytology [Fig. 2a], compared to 36% in the placebo group.

Tear inflammatory cytokines

A repeated-measures ANOVA showed that all nine tear cytokines significantly decreased in the O3FA group [Fig. 3]. For example, average IL-6 levels dropped at 6 months ($F [3, 147] = 6464.197$, $P < 0.001$). The Bonferroni-adjusted *post hoc* test confirmed a notable reduction in IL-6 from baseline to 3 months of dietary intervention (1424 ± 14.4 versus 1301 ± 16.8; $P < 0.05$). After 6 months, IL-6 fell further to 1191, which was significantly lower than both the pretreatment score and the 3-month measurement ($P < 0.001$). Repeated-measures ANOVA revealed no significant changes in tear cytokines including IL-6 at 6 months postintervention in the placebo group ($[3,147] = 0.515$, $P = 0.673$), and Bonferroni-adjusted *post hoc* analysis confirmed no significant differences from baseline to 1, 3, or 6 months. In contrast, all cytokines showed significant changes at 6 months in the O3FA group [see Tables 2 and 3].

Omega-3 index

In the O3FA group, repeated-measures ANOVA indicated that there was a significant change in mean omega-3 index over 6 months of intervention ($F [3, 147] = 6160$, $P < 0.001$). The *post hoc* test adjusted for multiple comparisons revealed that the O3FA dietary intervention did not result in a significant increase ($P < 0.001$) in mean omega-3 index from baseline to 1 month (ANOVA, $P = 0.823$) and baseline to 3 months (ANOVA, $P = 0.246$) of intervention, respectively (mean scores of 3.48 ± 0.7, 3.54 ± 0.7, and 3.70 ± 0.4, respectively). After 6 months of intervention, the omega-3 index increased to 5.8, which was significantly different ($P < 0.001$) from the score before treatment and after 3 months. In the placebo group, the mean change in omega-3 index was not significant over 6 months of intervention ($F [3, 147] = 540$, $P = 0.245$).

Table 1: Key characteristics (baseline)

*Measure	Group 1 (Omega 3)	Group 2 (Placebo)	*t*-test *P*
Age (years)	25.1±1.7	25.8±1.6	0.066
IL1β	513.7±45.7	510±45.4	0.781
IL2	136.8±4.9	138±5.3	0.090
IL4	374.9±4	376±4.5	0.093
IL5	277±3.8	278±4.2	0.141
IL6	1424±102	1418±96	0.057
IL8	481.5±2.7	482.7±3.4	0.064
IL10	467.2±3.7	471±4.2	0.116
IF γ	334.7±3.5	33±4	0.449
TNFα	330±3	334±2.8	0.055
DESS Score	7.4±1.2	7.6±1.5	0.073
Schirmer	17.3±1.8	17.5±1.9	0.548
TBUT	11.8±2	12.1±2.3	0.467
Nelson Grade	1.20±0.24	1.25±0.26	0.285
GCD (cells/mm²)	820±27	788±24	0.123

The data are presented as means±standard deviation, TBUT (tear film breakup time), and GCD (goblet cell density, cells/mm²)

Table 2: Mean test values for Group 1 (Omega-3) at baseline as well as at 3 months and 6 months postintervention

*Measure	Baseline	3 months	6 months	*P*
IL1β	512.7±45.7	452.7±46	392.5±45	<0.001
IL2	135.8±4.9	96.8±4.8	66.8±5	<0.001
IL4	374.9±13.9	323.8±14	273±14	<0.001
IL5	276.8±3.8	225±4	181±4.2	<0.001
IL6	1424±4.5	1304±16.8	1191±15.4	<0.001
IL8	481.5±2.7	431±5	341±3	<0.001
IL10	467±4	411±6	357±5	<0.001
IF γ	333.7±13.5	291±13	249.7±13.4	<0.001
TNFα	334±12.9	390±12	240±12	<0.001
DESS Score	7.4±1.2	2.4±0.8	1.2±1	<0.001
Schirmer	17.3±1.8	21.2±1.4	22.2±1.4	<0.001
TBUT	11.8±2	15.3±2	19.3±2.2	<0.001
Nelson Grade	1.2±0.25	0.59±0.29	0.38±0.29	<0.001
GCD (cells/mm²)	820±17	880±22.5	959±22	<0.001

*The data are presented as mean±standard deviation, TBUT (tear film breakup time), and GCD (goblet cell density, cells/mm²)

Conjunctival impression cytology

Nelson grade: Repeated-measures ANOVA in the O3FA group indicated a significant difference in the mean Nelson grade ($F [3, 147] = 10707.311$, $P < 0.001$) at 6 months. The *post hoc* test, after adjusting for multiple comparisons, showed that the O3FA intervention did not result in a significant change ($P = 0.650$) in the Nelson grade from pretreatment to 1 month of intervention (mean score of 1.2 ± 0.3 vs. 1.19 ± 2.8). After 6 months of intervention, the Nelson grade was reduced to 0.39, which was significantly different ($P < 0.001$) from the score before treatment and after 3 months. In the placebo group, the mean scores for the Nelson grade were not significantly different ($F [3, 147] = 1.926$, $P = 0.128$).

Table 3: Mean test values for Group 2 (Placebo) at baseline as well as at 3 months and 6 months postintervention

*Measure	Baseline	3 months	6 months	*P*
IL1β	510±45.4	508±44	504±46	0.133
IL2	138±5.3	136±6	134±6.4	0.298
IL4	376±4.5	376±4	375±5	0.660
IL5	278±4.2	274±5	272±4.4	0.877
IL6	1418±96	1418±90	1413±92	0.199
IL8	482.7±3.4	480±4	480±4.2	0.108
IL10	471±4.2	470±4	469±4	0.320
IF γ	336±4	334±3.8	331.8±4	0.373
TNFα	334±2.8	332±3.4	330±3.2	0.472
DESS Score	7.6±1.2	7±2	6.9±1.5	0.099
Schirmer	17.5±1.9	17.8±2	17.4±2.2	0.397
TBUT	12.1±2.3	12.4±2	12.8±2.4	0.620
Nelson Grade	1.25±0.26	1.22±0.2	1.2±0.4	0.876
GCD (cells/mm^2)	788±24	796±30	801±26	0.285

*The data are presented as mean±standard deviation, TBUT (tear film breakup time), and GCD (goblet cell density, cells/mm^2)

Table 4: Average test values comparing the two groups 6 months following intervention

*Parameter	Group 1 (omega 3)	Group 2 (Placebo)	*P* (independent *t*-test)
IL1β	392.5±45	504±46	<0.001
IL2	66.8±5	134±6.4	<0.001
IL4	273.9±14	375±5	<0.001
IL5	181±4.2	277±4.4	<0.001
IL6	1191±15.4	1413±92	<0.001
IL8	341±3	480±4.2	<0.001
IL10	357±5	469±4	<0.001
IF γ	249.7±13.4	331.8±4	<0.001
TNFα	240±12	330±3.2	<0.001
DESS Score	1.2±1	6.9±1.5	<0.001
Schirmer	22.2±1.4	17.4±2.2	<0.001
TBUT	19.3±2.2	12.8±2.4	<0.001
Nelson Grade	0.38±0.29	1.2±0.4	<0.001
GCD (cells/mm^2)	959±22	801±26	<0.001

*Expressed as mean±standard deviation, TBUT (tear film breakup time), GCD (goblet cell density (cells/mm^2)

Goblet cell density

A repeated-measures ANOVA in the O3FA group showed a significant difference in mean goblet cell density (F [3, 147] = 16584.828, $P < 0.001$) at 6 months [Fig. 2b]. The *post hoc* test revealed no significant change from pretreatment to 1 month ($P = 0.076$), with densities of 790 ± 22.5 vs. 820 ± 20. After 6 months, GCD increased to 960 cells/mm2, significantly different from pretreatment and 3 months ($P < 0.001$). In the placebo group, no significant differences were observed (F [3, 147] = 1.836, $P = 0.143$).

Dry eye symptoms

In the O3FA group, repeated-measures ANOVA showed significant differences in mean symptom scores (F [1.29, 63.367] = 563.081, $P < 0.001$) at 6 months. *Post hoc* analysis revealed a significant reduction ($P < 0.001$) in symptoms from baseline to 3 months (mean score: 7 ± 1.2 vs. 2.4 ± 0.8). By 6 months, the symptom score dropped to 0.3, significantly different ($P < 0.005$) from both baseline and 3 months. In contrast, the placebo group showed no significant change in mean symptom scores (F [3, 147] = 3.366, $P = 0.073$).

Between group comparisons

Table 4 shows the mean test values for Groups 1 and 2 after 6 months of dietary intervention. Significant differences ($P < 0.05$) were found in tear cytokines and GCD in patients taking omega-3 supplements. Group 1 also had notable improvements in dry eye symptoms, Schirmer test scores, and TBUT ($P < 0.001$). The placebo group (Group 2) improved in dry eye symptoms, TBUT, and Schirmer scores but showed no significant changes in tear cytokines or GCD.

Correlations

An inverse and significant (r = -0.924, $P < 0.0.001$) correlation was found between tear cytokine (IL-6) and GCD in Group 1 at 6 months. The correlation between tear cytokine (IL-6) and GCD was not significant at 6 months postintervention in the placebo group (r = -0.050, $P = 0.468$).

Discussion

In our multicenter trial, participants with an omega-3 index below 4% who took 2000 mg of omega-3s daily for 6 months had lower tear cytokine levels, a higher goblet cell density, and improved dry eye symptoms; the placebo group saw no significant changes.

After 6 months, the O3FA group's omega-3 index increased by 60%, reflecting high adherence. Baseline omega-3 levels were significantly and inversely associated with dry eye symptoms and tear cytokines ($P < 0.001$).

There is limited research on the effects of omega-3 supplementation in individuals with dry eye who have a low omega-3 index. A randomized controlled trial among adults with chronic dry eye found that participants with an omega-3 index below 4% experienced greater improvements in dry eye symptoms and goblet cell density than those with an index above 4%.[8] The study did not measure tear cytokines. A study by Bilinski *et al.*[12] found that healthy individuals with an omega-3 index above 5% who took 160mg EPA and 80mg DHA daily for 12 weeks saw a 10.1% increase in their omega-3 index. These studies suggest O3FAs are more effective in those with a low omega-3 index.

In laboratory settings, O3FAs are converted into anti-inflammatory compounds known as resolvins and protectins. These compounds are associated with a reduction in inflammation by lowering the production of proinflammatory cytokines in various parts of the body, including the eyes.[13]

Wang *et al.*[14] performed a meta-analysis of 19 RCTs involving 4246 patients with DED from various causes. The results show omega-3 FAs have therapeutic potential, despite differences in patient characteristics across studies. This study acknowledged the limitation that dietary practices vary significantly among Asian, American, and Mediterranean regions. In the subcontinent, especially in northern areas, cold-water fish is not commonly included in the local diet. Regional species such as Rohu, Catla, Pangas, and Magur have statistically lower ($P < 0.05$) omega-3 content compared to Salmon, Tuna, Sardines, and Mackerel.[15] A predominantly vegetarian diet that includes limited omega-3 sources from dark-green leafy vegetables and soybean oil may not provide adequate O3FA to raise the omega-3 index.

A direct comparison between the present study and other placebo-controlled trials is constrained by several factors.[16-18] Notably, these previous studies did not assess either omega-3 index or tear cytokine levels. Furthermore, the DREAM trial was conducted within an American cohort whose dietary habits differ from those of Asian populations.[19]

Roy *et al*.[20] conducted a study to evaluate the effects of 12-month omega-3 supplementation on tear cytokines (IL-1β, IL-6, IL-8, IL-10, IL-17A, IFN-γ, and TNF-α) in individuals with dry eye disease. Participants were randomly assigned to receive daily eicosapentaenoic and docosahexaenoic acids or a placebo (olive oil). At 6 months, a statistically significant difference between treatment groups was observed in the percent change from baseline concentrations of IL-6 and IL-8. No significant differences were noted between the two groups for IL-1β, IL-10, IL-17A, IFN-γ, and TNF-α at either 6 or 12 months. However, omega-3 index was not considered. In contrast, our study noted a significant reduction in all 9 measured cytokines at 6 months. These observations further substantiate that omega-3 index may serve as a valuable predictor for identifying individuals with dry eye who are most likely to benefit from oral omega-3 interventions.

An *in vitro* study utilizing cultured human corneal epithelial cells was conducted to investigate the therapeutic potential of O3FA supplementation for dry eye syndrome. The results demonstrated that O3FA supplementation enhanced cell viability and led to a marked reduction in inflammatory cytokines, with IL-6 and IL-8 levels decreasing by 50% ($P < 0.01$).[21] This *in vivo* study found over a 70% reduction in all measured cytokines (IL-1β, IL-6, IL-8, IL-10, IL-17A, IFN-γ, TNF-α) among dry eye patients with a low omega 3 index. In contrast, a 3-month open label study reported no significant decrease in IL-1β, IL-2, or TNF-α following O3FA and antioxidant supplementation.[22]

Current data suggest that maintaining O3FA supplementation for at least 6 months can maximize therapeutic benefits. This finding is supported by multiple trials in the subcontinent, where positive outcomes were observed when O3FA therapy lasted 6 months or more.[8,9]

There are some limitations to this trial. Due to cost constraints, only 102 patients were recruited for the study, which may have resulted in a type II error and overestimation. For impression cytology, the mRNA levels of IL-1β, IL-6, IL-8, and TNF-α were evaluated in a limited number of patients (n = 10). Second, it was not possible to assess the presence of omega-3 fatty acids in tears.

Conclusion

This randomized study underscores the potential advantages of oral O3FA supplementation in decreasing tear inflammatory cytokines in dry eye patients with an omega-3 index below 4%.

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