Oral Omega-3 Fatty Acid Treatment for Dry Eye in Contact Lens Wearers

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Purpose: The aim of this study was to evaluate the effect of dietary omega-3 fatty acid (O3FA) supplementation on dry eye symptoms, tear film tests, and conjunctival impression cytology in patients with contact lens wear–associated dry eye.

Methods: In this randomized, double-blind, multicentric trial, contact lens wearers (n = 496) were randomized to receive either O3FAs or placebo capsules (corn oil) twice daily for 6 months. Subjects underwent examinations at baseline, 3 months, and 6 months. At each visit, a questionnaire of dry eye symptoms and lens wear comfort was administered. Subjects further underwent measurement of tear film break-up time (TBUT) and a Schirmer test. Conjunctival impression cytology was performed by the transfer method. Improvement in symptoms and lens wear comfort were primary outcome measures. Changes from baseline in TBUT, Schirmer, and Nelson grade at 6 months were secondary outcome measures.

Results: The mean improvement in symptom score in the O3FA group was 4.7 ± 2 (2.0) as compared with 0.5 ± 2 (0.9) in the placebo group ($P < 0.0001$). Lens wear comfort levels improved significantly ($P < 0.0001$) from baseline. There was a significant increase in TBUT [3.3 ± 2 (1.5)] and Nelson grade [0.7 ± 2 (0.6)] in the O3FA group ($P < 0.0001$) as compared with 0.3 ± 2 (0.6) and 0.1 ± 2 (0.4) in the placebo group ($P = 0.164$ and 0.094, respectively). However, the magnitude of increase in Schirmer score [2.0 ± 2 (1.5)] was relatively small ($P = 0.08$).

Conclusions: The results of this study point toward benefits of orally administered O3FAs in alleviating dry eye symptoms, improving lens wear comfort, and cytological changes in contact lens wearers.

Key Words: contact lens, conjunctival impression cytology, dry eye syndrome, omega-3 fatty acids

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Contact lenses on the ocular surface are in proximity to the eyelid margin and tarsal conjunctiva on the front side and to the corneal epithelium, limbus, and bulbar conjunctiva on the back side, being separated by a pre- or post-lens tear film, respectively. Prolonged presence of a contact lens on the anterior corneal surface reduces the thickness of the prelens lipid layer and increases the tear evaporation rate.[1,2] Dryness of eyes is quite frequent in contact lens users; it may be accompanied by ocular discomfort, dissatisfaction, and inability to wear a contact lens for a desired period.[3] Moreover, prolonged lens wear may lead to a decrease in conjunctival goblet cells and squamous metaplasia of conjunctival epithelial cells.[4–8] Various factors such as lens material, lens water content, concurrent use of visual display terminals, ambient humidity conditions, and lens wear time may influence these changes on the ocular surface. Thus, it may be a reasonable assumption that contact lens–related problems are reflective of the overall conjunctival health.

Conjunctival surface health may be assessed by conjunctival impression cytology (CIC), which allows cells to be harvested from the ocular surface noninvasively. CIC may detect early subtle changes undetected by routine tear function tests; many investigators are of the opinion that it can be the first-line diagnostic investigation for ocular surface disorders, and an outcome measure in clinical trials.[9,10]

O3FAs found in certain fish oils have been shown to be beneficial in other ocular conditions such as age-related macular degeneration in evidence-based studies.[11] However, safety and efficacy of O3FAs in contact lens wear–related dry eye have not been documented.

This study hypothesizes that oral O3FA supplementation does improve dry eye symptoms, lens wear comfort, goblet cell density (GCD), and epithelial cell morphology (as seen on CIC) and clinical markers such as Schirmer-1 test values and tear film break-up time (TBUT) in contact lens wearers, as compared with the administration of placebo (corn oil).

METHODS

A prospective, multicentric, randomized, double-blind interventional study was performed at 3 referral eye centers, in the Northern part of the Indian subcontinent. The trial was approved by the institutional review boards and the local ethics committee. Written informed consent was obtained from all patients willing to participate in the study based on the Helsinki protocol.

Inclusion Criteria

A survey was conducted in regional universities, medical schools, and information technology parks. Female contact lens users experiencing dry eye symptoms and lens

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wear discomfort were identified and invited to take part in the trial. Subjects were recruited based on their response to [Dry Eye Scoring System (DESS)], a questionnaire of dry eye–related symptoms (Table 1).[10,12–14] All participants were using monthly or daily-wear soft contact lens. The lens type worn was separated according to the US Food and Drug Administration (FDA) category. The average daily lens wear time was calculated as hours per day. The total lens wear time was calculated in years.

Exclusion Criteria

Patients with current ocular infection, history of laser in situ keratomileusis, allergic conjunctivitis, herpetic eye disease, diabetes, and liver diseases were excluded. Other exclusion criteria were pregnant or lactating mothers, HIV, and hepatitis B and C. Patients with inability to swallow soft gel capsules, who were on aspirin or anticoagulant therapy, and those who were allergic to fluorescein were also excluded. Systemic (tetracyclines and corticosteroids) or topical medications (other than artificial tear supplements) that could affect tear film or meibomian gland functions were discontinued before intervention. However, patients were instructed not to use artificial tear preparations, 2 hours before testing. Computer work was not allowed during the course of the study as concurrent use of visual display terminals may independently influence ocular surface changes.

Randomization, Masking, and Sample Size Calculation

To calculate the sample size and to compare the mean difference in symptom scores between the 2 groups, a pilot study was first done on 50 subjects. The mean decrease in symptom score in the omega-3 group was 0.8 and in the placebo group was 0.7, respectively. The common SD was 0.5. Assuming 1:1 randomization, 90% power ($\alpha = 0.05$), and a precision error of 5% to detect difference of 20% or more in symptom score between 2 groups, the estimated sample size in each group was calculated to be 237 (http://www.stat.ubc.ca~rollin/stats/ssize/b2.html).

Patients were randomly allocated to 1 of the 2 groups by a parallel assignment. The allocation codes were generated by a DOS-based software in the department of Community Ophthalmology. The codes were sealed in blue envelopes and were opened by health care personnel not involved in patient care. The O3FA group received a dosage of two 300-mg capsules, each containing 180 mg eicosapentaenoic acid and 120 mg docosahexaenoic acid, twice daily for 6 months. The placebo group received 2 capsules containing corn oil, twice daily for 6 months. The subjects were masked to the contents. The 2 types of capsules and packs were similar to each other. The subjects were instructed to return the bottles at a monthly visit, wherein 2 packs with 120 capsules each were provided to them. The subjects were instructed to take a normal diet, and not to take additional dietary supplements.

TABLE 1. Dry Eye Questionnaire and Scoring System (DESS)

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

Scores of 0 to 6 were mild, 6.1 to 12 were moderate, and 12.1 to 18 indicated severely symptomatic dry eye[12–14] (Adapted from Bhargava R. Laser Eye Clinic, Noida, India). Adaptations are themselves works protected by copyright. So in order to publish this adaptation, authorization must be obtained both from the owner of the copyright in the original work and from the owner of copyright in the translation or adaptation.

OUTCOME MEASURES

The primary outcome measures were decrease from baseline in subjective dry eye symptoms and lens wear discomfort at 6 months after intervention. A score of 0 to 3 was assigned to dry eye symptoms, such as ocular fatigue, blurring of vision, itching or burning, sandy or gritty sensation, and redness, respectively (DESS) (Table 1)—scores indicating absent (0), sometimes present (1), frequently present (2), and always present (3). DESS is assessed on a scale of 0 to 18, with higher scores representing dry eye severity. A symptom score of 0 to 6 represents mild, 6.1 to 12 moderate, and 12.1 to 18 severe dry eye. Lens wear comfort was graded on a specially designed scale from 0 to 6 (0 = no discomfort to 6 = severe discomfort). The secondary outcome measures were a change in the Schirmer-1 test values as a measure of tear production, TBUT as a measure of tear film stability, and CIC scores (Nelson grade) for cellular morphology and GCD.

OCULAR EXAMINATION AND TEAR FUNCTION TESTS

The participants were instructed to visit the dry eye clinic in the morning, and all the tests were performed at the same time of the day (between 10 AM and 12 PM) in a dimly lit room. Patients were instructed not to use artificial tear preparations, 2 hours before testing. At each visit, the subjects underwent a detailed ocular examination by an independent investigator (who was not a study surgeon, Krishan Sharma). This included a recording of corrected distance visual acuity and slit-lamp examination; this included assessment of lid margins, eye lashes, and meibomian gland orifices for any blockage or occlusion.

At each examination, subjects underwent tests of tear film characteristics such as Schirmer, TBUT, and CIC. Furthermore, the subjects were given a dry eye questionnaire at each visit. The independent investigator (K.S.) was masked to the information obtained from the questionnaire.

TBUT was performed 30 minutes after the removal of the contact lens. Excessive eyelid manipulation was avoided

at this stage because it may adversely influence the results. A sterile fluorescein strip containing 1 mg fluorescein sodium (Madhu Instruments, Delhi, India) was applied over the inferior bulbar conjunctiva. The strip was moistened with normal saline solution before application. The patient was instructed to blink naturally, without squeezing, several times to distribute the fluorescein. The tear film was observed at a slit lamp using cobalt blue filter. The interval between the last complete blink and the first appearance of a dry spot on the cornea was recorded with a timer. Three readings were taken in succession and averaged. The subject then waited for another 30 minutes, and a Schirmer test with anesthesia (0.4% oxybuprocaine hydrochloride) was performed with eyes closed.

A single examiner performed CIC and was masked to information obtained from the questionnaire. One eye of each patient was selected randomly for examination.

Technique

CIC was performed by the transfer method after anesthetizing the eye with 1 drop of 4% xylocaine.[15] The lacrimal lake at the inner canthus was dried with a cotton-tip applicator. A circular 0.2-μm filter paper measuring 13 mm in diameter (Sartorius, Gottingen, Germany) was grasped with a blunt-tipped forceps and applied over the inferior bulbar conjunctiva. CIC samples were obtained from the nonexposed conjunctiva to eliminate the influence of environment-related factors on the ocular surface in the exposed part. The paper strip was gently pressed with a glass rod held in the other hand. The filter paper was removed in a peeling fashion after 4 to 10 seconds, and the specimen was transferred to the laboratory for fixation (ethyl alcohol, formaldehyde, and glacial acetic acid in 20:1:1 volume ratio) and staining. Because of relative ease of handling, the filter paper was first placed on a glass slide with albumin paste to transfer the specimen to the slide, instead of working directly. However, loss of the material adhered to the filter can be considered a potential disadvantage. The filter paper was then removed from the slide, and the slide was labeled and numbered. The slide was kept at room temperature and stained with periodic acid–Schiff and counterstained with hematoxylin and eosin. The mounted slide was first examined under a light microscope with ×100 low-power field (×10 objective lens). After localization, cells were then analyzed with ×400 final magnification (×40 objective). At least 10 HPF were examined for goblet cells and epithelial cells. The number of goblet cells per HPF were marked and counted. Estimated GCD = number of goblet cells counted per HPF divided by the sampling area covered in square millimeters. Grading and scoring was performed by the criteria suggested by Nelson et al.[16] Nelson grades 0 and 1 were regarded as normal, whereas grades 2 and 3 were considered to represent abnormal cytology.

Statistical Analysis

Statistical analysis was performed using SPSS software (version 22, IBM Inc). Means of 2 normally distributed samples were compared using a paired *t* test. The data were represented as mean ± 2 SD. χ^2 tests were used for proportions. $P < 0.05$ was considered statistically significant. Analysis of variance was used when there were more than 2 variables (between the type of contact lens, baseline symptom score, and comfort levels, respectively). A correlation analysis (along with regression) was performed to study the relationship between lens wear time, mean TBUT, Nelson grade, and GCD values at baseline and after 6 months of intervention, respectively. Pearson correlation coefficient, $R^2 > 0.5$ was significant.

RESULTS

A total of 496 contact lens users were enrolled in the study. Two hundred forty patients were randomized to the O3FA group and 256 patients to the placebo group, respectively. The mean contact lens wear time in the O3FA group was 3.1 ± 2 (0.91) years and in the placebo group was 3.2 ± 2 (1.6) years (range, 1.4–4). Noncompliance and gastric intolerance to O3FAs were the main reasons for dropout of 14 patients. All dropouts were included for analysis based on the last-observation-carried-forward method. However, 8 patients were excluded because of faulty impression cytology slides. The mean age of subjects in the O3FA group was comparable with the placebo group (*t* test, $P = 0.220$) (Table 2).

In the O3FA group, 22.3% of patients were mildly symptomatic, 72.7% moderately, and 5% severely symptomatic at baseline. At 6 months after intervention, 18% were

TABLE 2. Comparisons of Mean Test Values Between Treatment and Placebo Groups

	O3FA Group			Placebo Group		
Parameter	**Baseline**	**6 Months**	***P***	**Baseline**	**6 Months**	***P***
Symptom score	7.9 ± 2.3	3.3 ± 2.4	<0.001	7.7 ± 2.2	7.2 ± 2.2	0.010
TBUT, s	9.7 ± 1.6	13 ± 1.6	<0.001	9.5 ± 2.0	9.8 ± 2.3	0.164
Schirmer, mm	14 ± 4.5	16.1 ± 4.4	0.008	13.3 ± 4.6	13.5 ± 4.5	0.667
Nelson grade	2.2 ± 0.8	1.5 ± 0.6	<0.001	2 ± 0.9	1.88 ± 0.87	0.094
GCD (cells/mm^2)	892 ± 338	1051 ± 279	<0.001	875 ± 383	902 ± 374	0.449
Comfort level	3.1 ± 1	1.6 ± 0.8	<0.001	3.2 ± 1	3.1 ± 0.9	0.135

Values expressed as mean ± 2 SD.

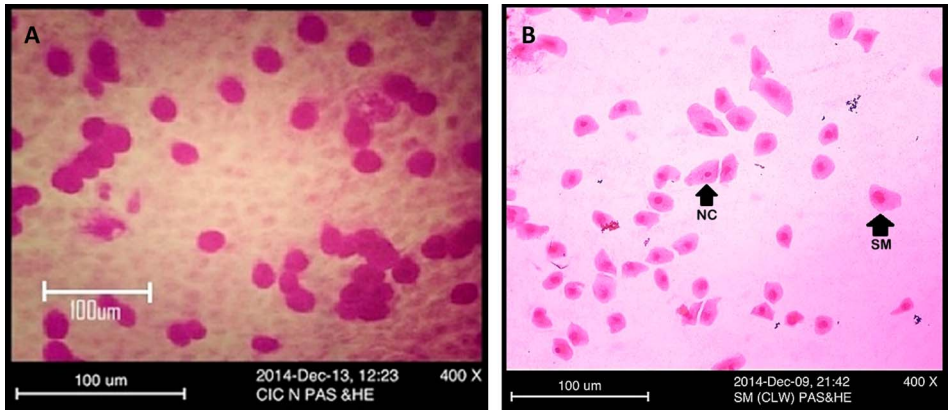

FIGURE 1. A, Photomicrographs of impression cytology specimens, stained with Periodic acid–Schiff stain (PAS) and hematoxylin and eosin (HE) at ×400 overall microscopic magnification showing high goblet cell count. B, Photomicrographs of impression cytology specimens, stained with PAS and HE at ×400 with squamous metaplasia. Arrows show a normal cell (NC) and increased nuclear–cytoplasmic ratio (SM), respectively.

asymptomatic, 76.5% mildly symptomatic, and 5.5% moderately symptomatic in the O3FA group.

In the placebo group, 34.4% were mildly symptomatic, 61.7% moderately, and 3.8% severely symptomatic at baseline. At 6 months after intervention, 41.2% were mildly symptomatic, 56.3% moderately, and 2.5% severely symptomatic in the placebo group.

Table 2 compares the test values at baseline and at 6 months after the start of dietary supplementation in the O3FA and placebo groups, respectively. At 6 months, there was a significant improvement ($P < 0.001$) in the symptom score, Schirmer, and TBUT in the O3FA group. However, improvement was not significant in the placebo group (paired t test, $P = 0.010$, 0.667, and 0.164, respectively).

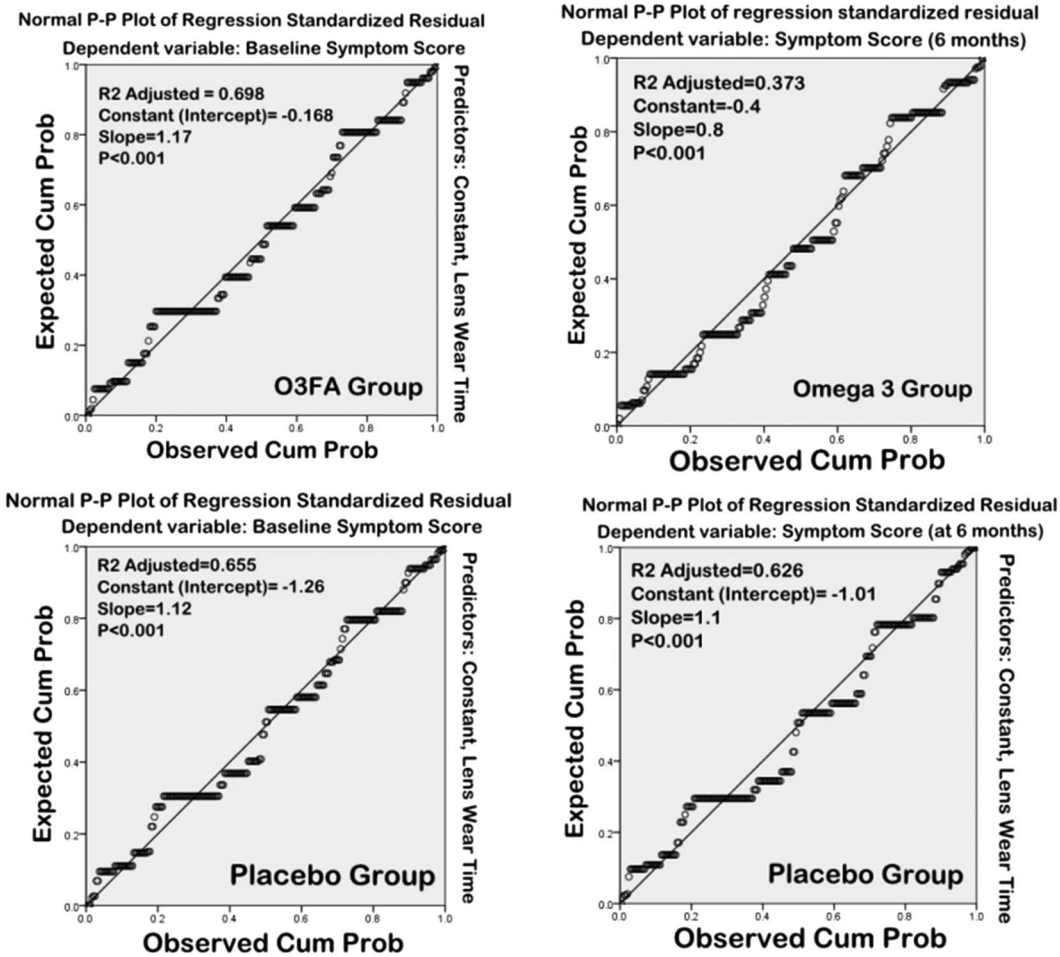

FIGURE 2. Normal probability plot of regression standardized residual; dependent variable symptom score with lens wear time.

Distribution and Variability in GCD Estimates at Baseline

The mean GCD in eyes with a Nelson grade 3 was 98 ± 2 (13.8); Nelson grade 2 had a GCD of 401 ± 2 (98), Nelson grade 1 had a GCD of 920 ± 2 (75), and in eyes with Nelson grade 0, the GCD was 1290 ± 2 (175), respectively. The field by field variability (with 10 different areas of the slide counted) for specimens with high goblet cells (Fig. 1A) was approximately 24 to 54 goblet cells per HPF (a variation of approximately 29%), whereas in specimens with low goblet cell counts (Fig. 1B), the variability was 1 to 5 goblet cells per HPF (a variation of about 68%).

At 6 months (Table 2), there was a significant improvement ($P < 0.001$) in the Nelson grade and GCD in the O3FA group. However, the improvement was not significant in the placebo group (paired t test, $P = 0.094$ and $P = 0.449$, respectively).

There was no significant difference in symptom severity and lens wear discomfort between the type (FDA types) of contact lens (AVOVA, $P = 0.278$ and 0.245, respectively). However, overall lens wear comfort improved significantly in the O3FA group (paired t test, $P < 0.0001$) as compared with the placebo group (paired t test, $P = 0.135$).

A correlation analysis between dry eye symptoms and lens wear time (at baseline) revealed that the 70% variability in the O3FA group (Pearson $R^2 = 0.698$) and 65% variability in the placebo group ($R^2 = 0.665$) could be explained by lens wear time. On analysis of variance, the probability corresponding to the $F < 0.0001$, suggests that there was less than 0.01% risk in assuming that the null hypothesis (no effect of lens wear time) was wrong. On linear regression (Fig. 2), there was a greater drift in slope of the normal probability plot of symptom score after dietary intervention in the O3FA group (1.2–0.8) as compared with the placebo group (1.1–1.1).

On correlation analysis (with regression) of Nelson grade with lens wear time, there was a greater drift in slope of the normal probability plot after dietary intervention in the O3FA group from (0.4–0.2) as compared with the placebo group (0.5–0.4) (Fig. 3); for GCD, the slope of the probability plot changed from 172 to 135 in the O3FA group as compared with a change from 192 to 185 in the placebo group (Fig. 4). The slope of the probability plot of regression of TBUT changed from 0.7 to 0.4 in the O3FA group as compared with a change from 0.7 to 0.8 in the placebo group (Fig. 5).

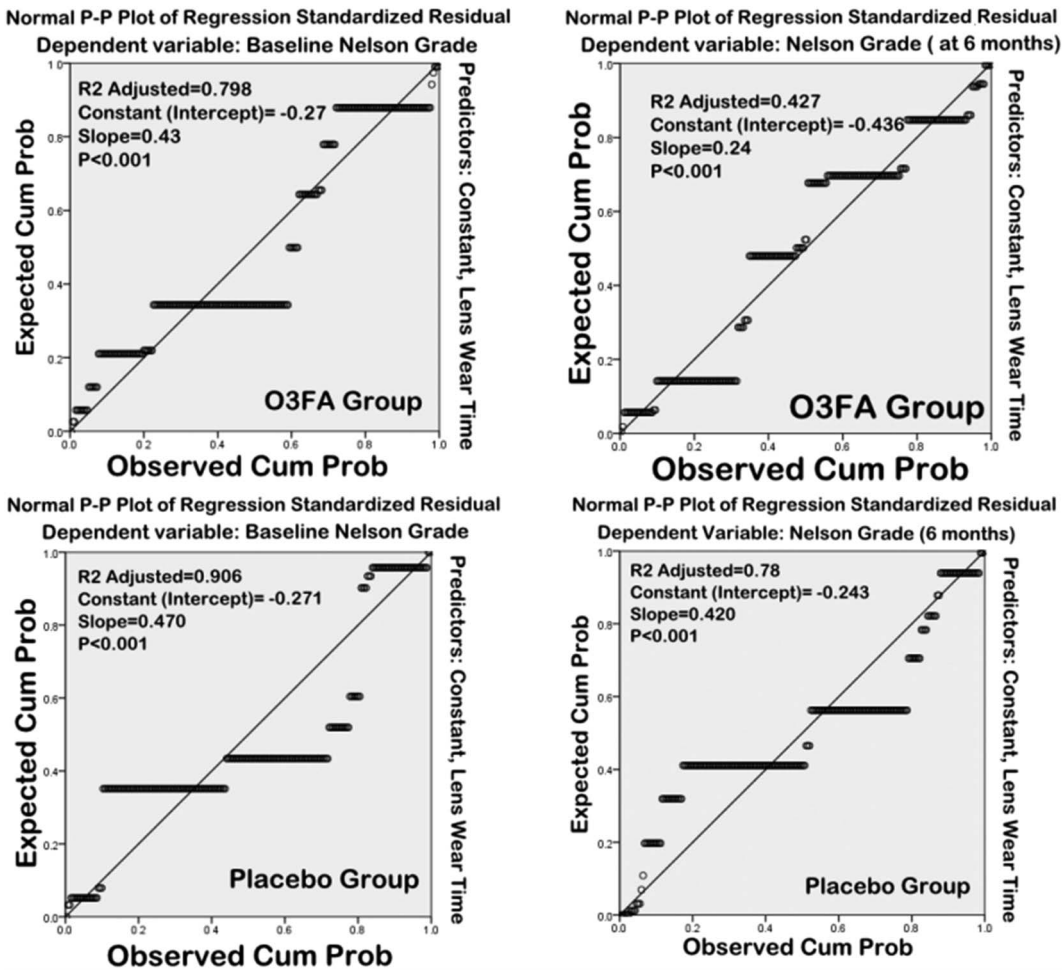

FIGURE 3. Normal probability plot of regression standardized residual; dependent variable Nelson grade with lens wear time.

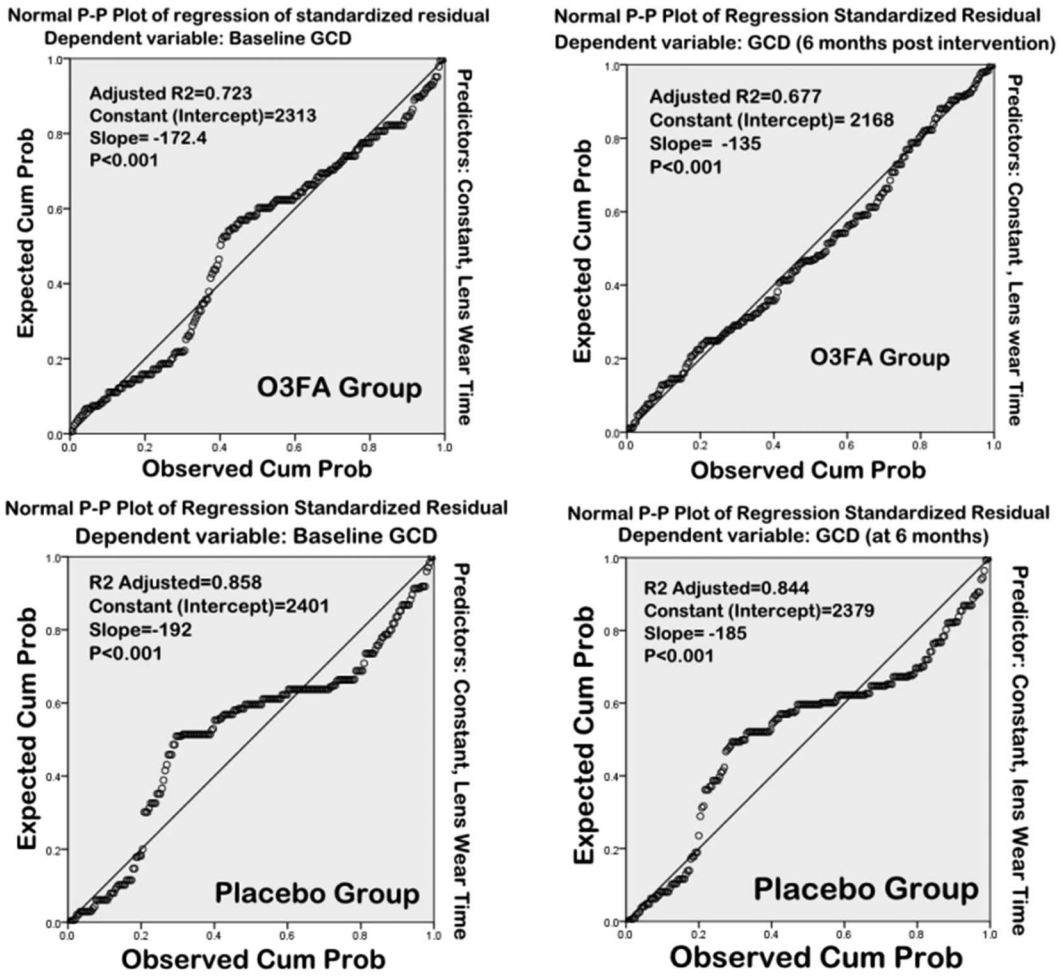

FIGURE 4. Normal probability plot of regression standardized residual; dependent variable GCD with lens wear time.

DISCUSSION

This study found that dietary supplementation with O3FAs for 6 months improved dry eye symptoms and lens wear comfort in contact lens wearers in comparison with the administration of a placebo (corn oil). This was associated with a significant increase in tear film stability (TBUT) in the O3FA group ($P < 0.001$). Tear production also increased (Schirmer test); however, the magnitude of improvement was relatively small ($P = 0.008$) (Table 2). Clinically, these findings were supported by a reduction in the number of blocked meibomian gland ducts in the O3FA group.

Recent studies have demonstrated subclinical inflammation in contact lens wearers, based on polymorphonuclear leukocytes, tear cytokine profile, and expression of inflammatory markers such as HLA-DR and ICAM-1 on the ocular surface epithelium, which may be correlated with dry eye severity and corneal surface staining.[17]

Essential fatty acids (EFA) are necessary for health but cannot be synthesized in the body and have to be obtained from dietary sources. The 2 main EFAs are the 18 carbon omega 6 and omega-3 fatty acids. Omega 6 fatty acids are proinflammatory and produce mediators such as prostaglandin E2 and leukotriene B4. In contrast, omega-3 fatty acids such as eicosapentaenoic acid block synthesis of these lipids and also interleukin 1 and tumour necrosis factor-alpha. The ratio of these 2 EFAs determines the overall inflammatory status of the body.[18–20]

In Western countries, the ratio of O6FA to O3FA is approximately 15 to 16:1, which is strikingly high.[21] Fish are a rich source of polyunsaturated fatty acids, particularly, O3FAs, which are beneficial for health. This study was conducted in the Northern part of the Indian subcontinent where the diet is predominantly vegetarian; cold water fish is not an essential component of the diet as compared with the diet of coastal and Southern India. Therefore, one must also take into account the fact that common Indian fishes such as Rohu, Catla, Pangas, and Magur have a significantly lower O3FA content, in comparison with Salmon, Tuna, Sardines, and Mackerel.[22] Hence, in the case of North Indian populations, the point of consideration is the fact that small quantities of omega-3 alpha linoleic acid, obtained from dark green leafy vegetables and soybean oil in vegetarians, are unlikely to provide acceptable O3FA levels in these subjects.

In a randomized, double-masked placebo-controlled trial in female contact lens users (n = 76), Kokke et al found that oral administration of evening primrose oil (O6FAs) for 6

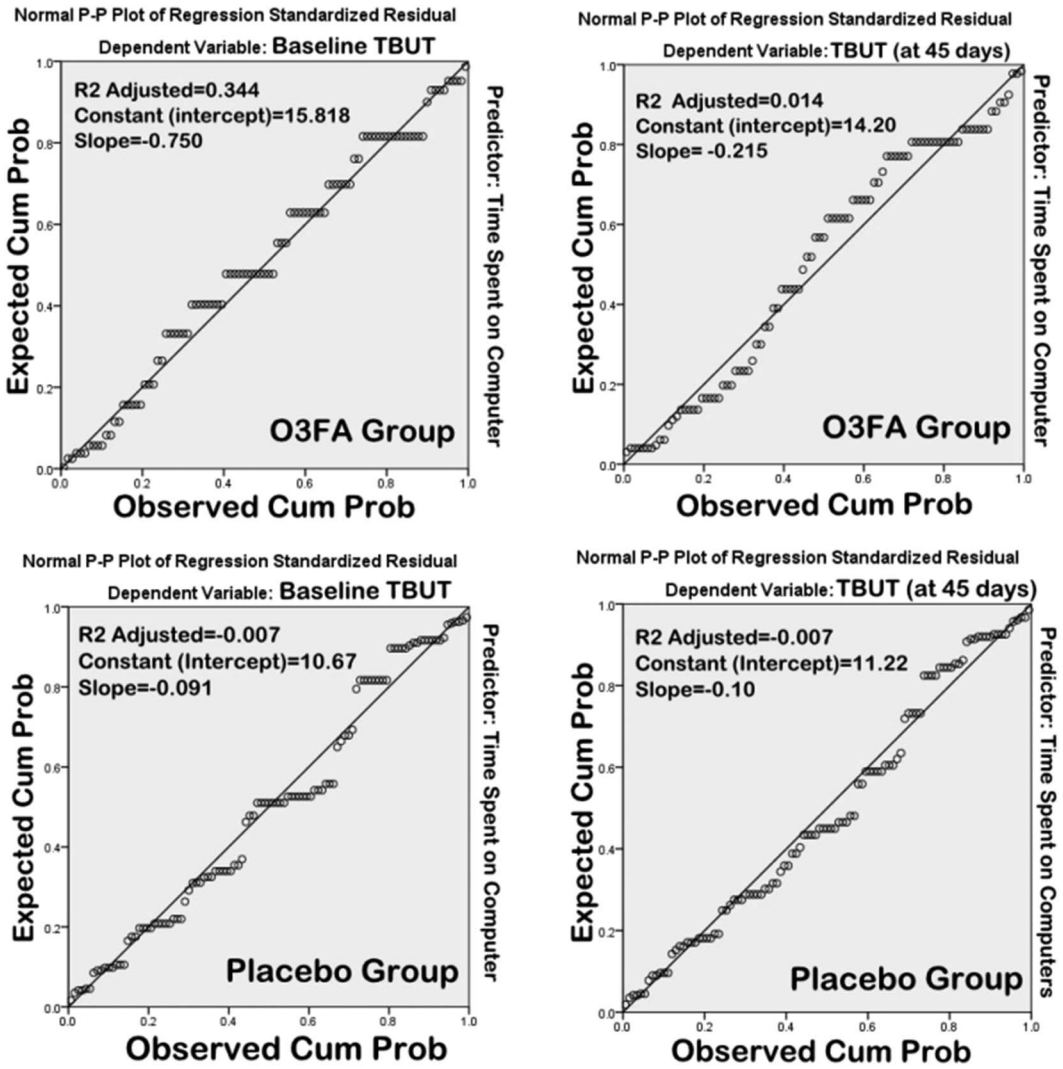

FIGURE 5. Normal probability plot of regression standardized residual; dependent variable TBUT with lens wear time.

months was associated with a significant improvement in symptoms and lens wear comfort, along with an increase in the tear meniscus height in the test group. The authors attributed these changes to a reduction in ocular surface inflammation and increased tear production, despite the fact that O6FAs are proinflammatory; it is possible that O3FAs and O6FAs need to be administered together within a reasonable ratio for an effective antiinflammatory action.[23,24]

In this study, O3FA dietary intervention significantly improved epithelial cell morphology and GCD (Nelson grade) in the O3FA group; however, a small improvement was also seen in the placebo group. The placebo used in this study was corn oil; it contains proinflammatory linoleic acid (O6FA) and small quantities of oleic acid (O9FA). Therefore, the possibility of influence of corn oil on dry eye symptoms (worsening), lens wear comfort, tear film tests, and ocular surface changes cannot be ruled out. Moreover, deviations from inertness of a placebo could also bias comparisons when the absolute treatment effect is small.[25] However, this deviation from inertness resulted in a positive influence (betterment) on dry eye symptoms and Nelson grade (0.1) (although this did not reach the 1% level of significance), and indirectly, skewed the results in favor of O3FAs. However, there was no improvement in lens wear comfort at 6 months.

The shortcoming of this study was that GCD estimates were higher as compared with other studies. Goblet cell distribution differs across CIC specimens, depending on the sampling area used. Kessing[26] studied GCD in normal subjects and found a density of 1599 goblet cells per square millimeter in the inferior bulbar conjunctiva, in contrast to 400 cells per square millimeter in interpalpebral part; goblet cells were least dense in the superior bulbar part. Recently, Doughty sampled bulbar conjunctiva of normal subjects and found a tendency toward higher goblet cell values in samples taken from normally covered locations (inferior and superior bulbar conjunctiva) of the open eye (at 973 $\pm$ 789 cells/mm^2) than in samples taken from exposed (interpalpebral) locations (at 427 $\pm$ 376 cells/mm^2). Furthermore, the use of a small

sampling area (high-power field of view) is likely to result in an unacceptably large uncertainty (variability) in the GCD estimates.[27,28] In this study, CIC samples were obtained from nonexposed part of the conjunctiva (inferior bulbar) and GCD was estimated at ×400 (small sampling area) overall magnification (although 10 different regions of the slide were examined). This could probably account for the higher goblet cell counts and density obtained in CIC specimens in this study.

Currently, there are no formal recommendations (dose and duration) or FDA-approved formulations for dietary consumption of EFAs in the treatment of dry eye disease. However, most published studies consistently report symptomatic improvement in patients with dry eye after dietary supplementation of O3FAs, but there is variability regarding improvement in tear film tests.[29–32] Small sample size and different treatment protocols (difference in opinion regarding dose, duration, and combination of O3FAs/O6FAs) in most of these studies could probably account for the variability in results.

In conclusion, the results of this study highlight the beneficial effects of orally administered O3FAs in decreasing dry eye symptoms and improving lens wear comfort in patients with contact lens–related dry eye. The antiinflammatory effects of O3FA seem to be the likely reason.

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REFERENCES

1. Dillehay SM. Does the level of available oxygen impact comfort in contact lens wear?: A review of the literature. *Eye Contact Lens.* 2007; 33:148–155.
2. Rohit A, Willcox M, Stapleton F. Tear lipid layer and contact lens comfort: a review. *Eye Contact Lens.* 2013;39:247–253.
3. Sengor T, Aydin Kurna S, Ozbay N, et al. Contact lens-related dry eye and ocular surface changes with mapping technique in long-term soft silicone hydrogel contact lens wearers. *Eur J Ophthalmol.* 2012;22: S17–S23.
4. Doughty MJ. Objective assessment of squamous metaplasia of conjunctival epithelial cells as associated with soft contact lens wear versus non-lens wearers. *Cornea*. 2014;33:1095–1102.
5. Cakmak SS, Unlü MK, Karaca C, et al. Effects of soft contact lenses on conjunctival surface. *Eye Contact Lens.* 2003;29:230–233.
6. Tomatir DK, Erda N, Gürlü VP. Effects of different contact lens materials and contact lens-wearing periods on conjunctival cytology in asymptomatic contact lens wearers. *Eye Contact Lens.* 2008;34:166–168.
7. Pisella PJ, Malet F, Lejeune S, et al. Ocular surface changes induced by contact lens wear. *Cornea*. 2001;20:820–825.
8. Doughty MJ. Contact lens wear and the development of squamous metaplasia of the surface cells of the conjunctiva. *Eye Contact Lens.* 2011;37:274–281.
9. Lopin E, Deveney T, Asbell PA. Impression cytology: recent advances and applications in dry eye disease. *Ocul Surf.* 2009;7:93–110.
10. Kumar P, Bhargava R, Kumar M, et al. The correlation of routine tear function tests and conjunctival impression cytology in dry eye syndrome. *Korean J Ophthalmol.* 2014;28:122–129.
11. Merle BM, Benlian P, Puche N, et al; Nutritional AMD Treatment 2 Study Group. Circulating omega-3 fatty acids and neovascular age-related macular degeneration. *Invest Ophthalmol Vis Sci.* 2014;55: 2010–2019.
12. Kumar P, Bhargava R, Kumar M, et al. Dry eye syndrome: a diagnostic enigma. *Int J Contemp Surg.* 2013;1:72–77.
13. Bhargava R, Kumar P, Kaur A, et al. The diagnostic value and accuracy of conjunctival impression cytology, dry eye symptomatology, and routine tear function tests in computer users. *J Lab Physicians.* 2014;6: 102–108.
14. Bhargava R, Kumar P, Kumar M, et al. A randomized controlled trial of omega-3 fatty acids in dry eye syndrome. *Int J Ophthalmol.* 2013;6: 811–816.
15. Shrestha E, Shrestha JK, Shayami G, et al. The conjunctival impression cytology between the diagnosed cases of dry eye and normal individuals. *Nepal J Ophthalmol.* 2011;3:39–44.
16. Nelson JD, Havener VR, Cameron JD. Cellulose acetate impressions of the ocular surface. Dry eye states. *Arch Ophthalmol.* 1983;101: 1869–1872.
17. Rolando M, Barabino S, Mingari C, et al. Distribution of conjunctival HLA-DR expression and the pathogenesis of damage in early dry eyes. *Cornea*. 2005;24:951–954.
18. Rosenberg ES, Asbell PA. Essential fatty acids in the treatment of dry eye. *Ocul Surf.* 2010;8:18–28.
19. Calder PC. Polyunsaturated fatty acids, inflammation, and immunity. *Lipids*. 2001;36:1007–1024.
20. Roncone M, Bartlett H, Eperjesi F. Essential fatty acids for dry eye: a review. *Cont Lens Anterior Eye.* 2010;33:49–54.
21. Simopoulos AP. The importance of the ratio of omega-6/omega-3 essential fatty acids. *Biomed Pharmacother.* 2002;56:365–379.
22. Jakhar KA, Pal AK, Reddy DA, et al. Fatty acids composition of some selected Indian fishes. *Afr J Basic Appl Sci.* 2012;4:155–160.
23. Kokke KH, Morris JA, Lawrenson JG. Oral omega-6 essential fatty acid treatment in contact lens associated dry eye. *Cont Lens Anterior Eye.* 2008;31:141–146.
24. Harbige LS. Fatty acids, the immune response, and autoimmunity: a question of n-6 essentiality and the balance between n-6 and n-3. *Lipids*. 2003;38:323–341.
25. De Craen AJ, Tijssen JG, Kleijnen J. Is there a need to control the placebo in placebo controlled trials? *Heart*. 1997;77:95–96.
26. Kessing SV. Mucous gland system of the conjunctiva. A quantitative normal anatomical study. *Acta Ophthalmol (Copenh).* 1968;95:1.
27. Doughty MJ. Goblet cells of the normal human bulbar conjunctiva and their assessment by impression cytology sampling. *Ocul Surf.* 2012;10: 149–169.
28. Doughty MJ. Sampling area selection for the assessment of goblet cell density from conjunctival impression cytology specimens. *Eye Contact Lens.* 2012;38:122–129.
29. Creuzot C, Passemard M, Viau S, et al. Improvement of dry eye symptoms with polyunsaturated fatty acids [in French]. *J Fr Ophtalmol.* 2006;29:868–873.
30. Wojtowicz JC, Butovich I, Uchiyama E, et al. Pilot, prospective, randomized, double-masked, placebo-controlled clinical trial of an omega-3 supplement for dry eye. *Cornea*. 2011;30:308–314.
31. Oleñik A, Jiménez-Alfaro I, Alejandre-Alba N, et al. A randomized, double-masked study to evaluate the effect of omega-3 fatty acids supplementation in meibomian gland dysfunction. *Clin Interv Aging.* 2013;8:1133–1138.
32. Kangari H, Eftekhari MH, Sardari S, et al. Short-term consumption of oral omega-3 and dry eye syndrome. *Ophthalmology*. 2013;120: 2191–2196.