ACQUAINT PUBLICATIONS ....Disseminate your Research

Journal of Community Medicine and Public Health Reports ISSN: 2692-9899

Research article Volume 2 Issue 2

A Novel Liposome-Based, Aminoacid- and Vitamin-Containing Tear Substitute in Patients with Evaporative Dry Eye Disease. A Pilot Prospective Study.

Rocco Luigi Modugno[1], MD, Oren Mark Feuerman[1], MD, Alvise La Gloria Valerio[1], MD, Tania Scalora[1], MD, Elena Salami[1], MD, Andrea Leonardi[1, *], MD

[1]Department of Neuroscience, Ophthalmology Unit, University of Padua Via Giustiniani 2, 35128 Padua, Italy

[*]**Corresponding Author:** Andrea Leonardi, Department of Neuroscience, Ophthalmology Unit, University of Padua Via Giustiniani 2, 35128 Padua, Italy

Received date: 02 March 2021; **Accepted date:** 15 March 2021; **Published date:** 19 March 2021

Citation: Modugno RL, Feuerman OM, La Gloria Valerio A, Scalora T, Salami E, et al. A Novel Liposome-Based, Aminoacid- and Vitamin-Containing Tear Substitute in Patients with Evaporative Dry Eye Disease. A Pilot Prospective Study. J Comm Med and Pub Health Rep 2(2): https://doi.org/10.38207/jcmphr20210014

Abstract

Purpose. Dry eye disease (DED) is a common ocular disorder with a potentially severe impact on patients' quality of life. Tear substitutes are the first-line treatment. The aim of this pilot study was to collect preliminary data concerning the efficacy and safety of a new liposome-based tear substitute - Lacrisek Ofta Plus - enriched with L-proline, L-glycine, L-lysine hydrochloride, and L-leucine as well as vitamin A and E.

Methods. Sixteen random-chosen eyes from as many consecutive patients (8 men and 8 women, mean age 55.8±16.2 years) suffering from evaporative DED, confirmed by a positive InflammaDry test for MMP-9 pathologic levels, were treated using the investigational tear substitute for a 30-day period. The contralateral eye was treated with a different substitute whose use in the clinical setting was already documented. Changes in objective (tear film break-up time (TF-BUT), Oxford fluoresceine staining score, van Bijsterveld Lissamine green staining score, Meibomian glands score, InflammaDry test, conjunctival hyperemia) and subjective (Ocular Surface Disease Index (OSDI) score, burning, photophobia, foreign body sensation, itchy eyes, tearing) endpoints at the end of the treatment period were compared to those recorded beforetreatment.

Results. After 30 days, eyes treated with the investigational device showed a significant improvement in the TF-BUT time (5.27±2.20 vs. 4.27±2.40 seconds; p=0.007) and the Oxford fluoresceine staining score (1.82±0.98 vs. 2.27±0.90, p=0.019). Eyes positive to the InflammaDry test were significantly less (62.5 %) than those at baseline. Patients also reported a significant reduction of burning sensation at the treated eye. Conjunctival hyperemia showed a trend toward improvement. The OSDI and van Bijsterveld Lissamine green staining score, as well as other self-reported symptoms, did not change significantly. No patient suffered from any adverse effect.

Conclusions. Within the limitations of the present study, Lacrisek Ofta Plus seems to be safe and effective in managing symptoms of dry eye disease, consistently with the beneficial effects ascribed to its liposome-based, aminoacid- and vitamin-containing formulation. Further studies are necessary to confirm these preliminary results.

Keywords: evaporative dry eye disease, liposome-based tear substitutes, amino acid-based tear substitutes, tear film evaluation, MMP-9, vitamin A, vitamin E.

Introduction

The composition, integrity, and stability of the tear film can be adversely affected by multiple patient-related and environmental factors, resulting in the development of dry eye disease (DED), one of the most common ocular disorders. Both its incidence and prevalence are continuously growing **[1,2]**. Aging is one of the main contributors to tear film changes, with an incidence ranging from 8 % in subjects under 60 to 20 % in those over 80 **[3]**. However, the huge spread of digital devices, surgical refractive treatments, and contact lenses use are causing an increasing prevalence of DED among both young people and adults **[4]**. According to the revised definition provided by the second Tear Film and Ocular Surface Society Dry Eye Workshop (TFOS DEWS II) report **[5,6]**, DED is an ocular surface multifactorial disease, characterized by a disruption of tear film homeostasis. Etiology involves tear film instability and hyperosmolarity, ocular surface inflammation and damage, and neurosensory abnormalities. DED impacts patients' quality of life heavily, limiting daily activities (such as reading, using digital devices, driving, and watching television) **[5,7]** to such an extent that the patient may develop mood disorders like anxiety, depression, and frustration **[8]**. DED can be classified as aqueous deficient or evaporative. Evaporative DED is most common, accounting for about 50 % of cases **[5,9]**. Mixed forms are also quite common as they account for about one-third of cases **[9]**. Depending on symptoms and signs, DED can be further classified as mild, moderate, or severe **[10]**.

Biomicroscopic examination of the ocular surface, of the tear film, and the use of validated questionnaires (such as the Ocular Surface Disease Index - OSDI – questionnaire **[11]**) are the principal diagnostic tools for evaluating DED and its symptoms. Local inflammation markers, such as matrix metalloproteinases (MMPs), may also be quantified by adjunctive tests **[12]**. Metalloproteinase- 9 (MMP-9), an MMP produced by corneal epithelial cells, fibroblasts, leukocytes, and lacrimal gland cells, plays a role in the remodeling process of the extracellular matrix, acting in both normal corneal healing processes and pathological states (i.e., ocular allergy, keratitis, blepharitis, and DED). Tear hyperosmolarity, a core mechanism of DED pathogenesis, stimulates the over-expression of MMP-9, as well as that of other pro-inflammatory cytokines, which in turn promote inflammation and epithelial cell loss **[13-15]**. Tear substitutes are the current mainstay of DED therapy, regardless of the disease severity. Lipid-based substitutes are emulsions containing mineral oils, castor oil, olive oil, glycerin carbopol, lecithin, phospholipids, and saturated and unsaturated fatty acids. They are particularly suitable for treating evaporative DED since their composition is closer to that of the deficient tear lipid layer **[16]**. Liposomal eye drops formulations–made of bilayered spherical lipid vesicles encapsulating the aqueous-soluble material – have also been tested, showing favorable outcomes in managing DED, improving both subjective and objective endpoints [**17-21]**. To improve the dwell time and lubricating effect, carboxymethylcellulose and other agents can be used to increase viscosity, protecting the ocular surface, and increasing the density of goblet cells **[22]**. Tear substitutes may also incorporate free amino acids, given their role in protecting the tear film, such as proline and glycine, the main components of type I collagen - the most abundant protein in the corneal stroma - and lysine and leucine, which contribute to the spatial arrangement and assembly of the fibrils, and the transparency of the corneal stroma **[23]**. Tear substitutes may also be added with antioxidant agents, such as vitamin A (retinyl palmitate) and vitamin E (α-tocopherol) to contrast oxidative stress and the consequent inflammation that contributes to the establishment, maintenance, and progression of DED. In DED patients, vitamin A-based tear substitutes have been shown to increase the quality of vision and contribute to improving objective parameters, such as Tear Film Break-Up Time (TF-BUT), Schirmer's test score, rose bengal and fluorescein staining scores, as well as subjective DED symptoms **[24,25]**. Tear substitutes containing vitamin E have also been shown to be effective in managing DED as measured by the variation in the TF-BUT time as well as the OSDI and Schirmer test scores **[26]**. A new liposome-based ophthalmic solution containing L-proline, L- glycine, L-lysine hydrochloride, and L-leucine as well as vitamin A and vitamin E, (Lacrisek Ofta Plus, Sooft Italia S.p.A., Italy) has been recently placed on the market. Its formulation has been conceived to exploit both the benefits of liposome-mediated solution delivery and the protecting and anti-oxidative action of the other constituents, possibly enhancing its overall effectiveness in managing DED symptoms. At present, this tear substitute – to the authors' knowledge – has still not been investigated in the clinical setting. This pilot study, therefore, aims to gather preliminary evidence concerning its efficacy and safety.

Materials and methods

This pilot, prospective longitudinal cohort study included 16 eyes from as many consecutive patients (8 male and 8 females; age range 29-76 years; mean age 55.8±16.2 years) diagnosed with evaporative DED. Patients were enrolled among those who were referred to the ocular surface and cornea service of the University of Padua. The study was conducted following the principles outlined in the Declaration of Helsinki and the Good Clinical Practices concerning clinical investigations of medical devices for human subjects. The diagnosis of evaporative DED was established when at least one of the following was present: clinically proven Meibomian Gland Dysfunction (MGD), tear film break-up time (TF-BUT) < 5 seconds in at least 3 measurements, Schirmer test I > 3 mm in 5 minutes. Patients were included if their age was >18 years, they had a diagnosis of evaporative DED for at least 3 months, their OSDI score was at least 18 on the screening and enrollment visit (baseline), they showed a best-corrected visual acuity (BCVA) of at least 55 letters (ETDRS chart) and were positive to the InflammaDry test for pathologic MMP-9 levels. Patients were excluded if they declared they were using any lipid-based tear substitutes on the day of enrollment or had already been treated, over the previous 30 days, with eye drops containing benzalkonium chloride. They were excluded also if they were using contact lenses or punctal plugs over the 30 days before enrollment or had undergone eyelid hygiene at least 1 month before enrollment. Other exclusion criteria included women of childbearing age either pregnant or not using contraceptive methods, hypersensitivity to drugs under investigation, active ocular allergy, eyelid anomalies, corneal ulcers, keratoconus, corneal epithelial dystrophies, active infective keratitis, corneal neovascularization, history of herpetic keratitis, and ongoing treatment with systemic drugs that may be associated with DED (such as antihistamines, antidepressants, antipsychotics).

Objective and endpoints

The primary study aim was to collect preliminary evidence concerning the effectiveness of the Lacrisek Ofta Plus device in improving objective and subjective DED symptoms after 30 days of treatment (the null hypothesis being no difference would be observed). The corresponding objective primary endpoints were: the tear film break-up time (TF-BUT); scores describing the fluorescein and Lissamine green conjunctival and epithelial staining patterns, as well as the number of eyes positive to the InflammaDry test; the subjective primary endpoint was the self-reported Ocular Surface Disease Index (OSDI) score. The working hypothesis is that all these parameters would significantly change, compared to baseline, after 30 days of treatment. The study also primarily aimed to gather preliminary data concerning device safety, by assessing the frequency and nature of adverse events, if any would occur. TF-BUT was evaluated after instilling 2 µl fluorescein, as the mean of three consecutive measures per eye. The fluorescein staining pattern of the corneal and conjunctival epithelium was scored from 0 to 5 according to the modified Oxford scale **[27]**. Corneal and conjunctival staining was repeated using Lissamine green and scoring the corresponding pattern according to the van Bijsterveld 0-9 scale **[28]**. The OSDI is a 12-item questionnaire, whose questions are grouped into three sections, namely ocular symptoms, vision-related function, and environmental factors making eyes feeling uncomfortable. It allows patients to subjectively assess ocular symptoms of DED and how they impact their quality of life. Each item is assigned a score from 0 (the discomfort being present none of the time) to 4 (the discomfort being present all the time); the sum of scores allows to detect normal eyes or to stratify DED as mild, moderate, or severe **[11]**. The InflammaDry test (Rapid Pathogen Screening, Inc, Sarasota, FL, USA) is a rapid, disposable immunological test for semi-quantitative measurement of MMP-9, a nonspecific inflammatory marker that has consistently been shown to beelevated in the tears of patients suffering from DED. Normal MMP-9 levelsin human tears range from 3 ng/ml to 40 ng/ml **[13-15]**. The InflammaDry test returns a positive result if MMP-9 level is > 40 ng/ml, thus adjuvating in confirming a diagnosis of DED and pathologic ocular surface inflammation **[29,30]**. Adjunctive endpoints included: measuring Meibomian gland obstruction through a 0 to 3 score (0 = no obstructed glands; 2 = 1-2 obstructed glands; 2 = 3-4 obstructed glands; 3 = all glands obstructed); the doctor assessing conjunctival hyperemia and the patient self- reporting burning, photophobia, foreign body sensation, itchy eyes, and tearing by using a 0-4 scoring system (0 = absent, 1 = mild, 2 = moderate, 3 = severe, 4 = very severe). All eyes were also routinely scoped by biomicroscopic evaluation of the whole ocular surface.

Study design

As published clinical data concerning the application of the investigational tear substitute to manage DED are still lacking and given the study objective was that of collecting only preliminary information on its effectiveness and safety, the authors deemed it unethical to use it to treat all eyes. Accordingly, they envisaged a protocol involving one of the patient's eyes to be treated using the investigational device, and the other using a tear substitute (Optive Plus, Allergan, Ireland) containing sodium carboxymethylcellulose, glycerin, castor oil, polysorbate, levocarnitine, and erythritol whose safety and effectiveness have been proved in the clinical setting **[31,32]**. Similar concerns prompted the authors to limit the number of patients to be recruited and to not calculate any sample size. As the disease is not always symmetrical and eyes usually do not respond equally to therapy, and to avoid any experimenter's bias, eyes were randomized to receive either one or the other tear substitute.

Treatment and assessment

Patients were screened, enrolled, and provided their informed consent on a first visit (baseline), which included collecting and assessing their anamnesis; a routinely ocular surface biomicroscopic assessment; subjecting them to the InflammaDry and Schirmer tests, and having their TF-BUT measured. Their Meibomian gland obstruction and signs and symptoms were assessed as described in the Objective and endpoints section and they answered the OSDI questionnaire. Patients were instructed to use the tear substitutes according to their respective manufacturer instructions (coincidentally, both are to be administered at the same dosage of 1 drop 4 times a day) for 30 days as well as to refer to the treating clinician, either by e-mail or phone, should any adverse event occur. Before dismissal, they were scheduled to report to the center after 30 days for further assessment.

At the 30-day visit, they underwent routinely biomicroscopic examination and were subjected to the InflammaDry test. TF-BUT was measured, and patients answered the OSDI questionnaire. Meibomian gland obstruction assessment and signs and symptoms evaluation were carried out as on the first visit. Even if performing any comparison between the investigational device and the alternative substitute was explicitly out of the scope of the study, all evaluations at baseline and the 30-day control visit were carried out at both eyes for sake of completeness.

Statistical analysis

Given the altogether preliminary nature of the present study, no sample size calculation was performed. The patients' characteristics, as well as the endpoints of interest, were described by calculating their absolute and relative frequencies when discrete or categorical, or their median and interquartile (IRQ) range or mean and standard deviation (SD) when found to have a non-normal or normal distribution, respectively, by applying the Shapiro-Wilk test. To assess if the investigational tear substitute was effective in managing DED symptoms, the endpoints of interest collected at the 30-day visit were compared to those at baseline by means of parametric or non-parametric test for normal and non-normal variables, respectively, namely t-tests or Wilcoxon signed-rank tests for paired data. For sake of completeness, the same analyses were carried out concerning the eyes treated using the alternative tear substitute. Data analysis was performed using standard statistical software (SAS software, version 9.1.3, SAS Inc., Cary, NC). Values arepresented as mean ± standard deviation (SD). Test results were regarded as significant if $p < 0.05$.

Results

Sixteen patients, 8 women and 8 men, were enrolled, aged 55.8 ± 16.2 years (range: 29-76). Results are shown in **(Figure 1)**. At the eyes treated using the investigative tear substitute, the mean TF-BUT after thirty days of treatment, 5.27±2.20 seconds, was significantly higher than at baseline (4.27 ± 2.40 seconds; p = 0.007). The decrease in the Oxford score (1.82±0.98 at 30 days vs. 2.27±0.90 at baseline) was also statistically significant (p=0.019), while the van Bijsterveld staining score for Lissamine (3.00±2.10 at 30 days vs. 4.10±1.80 at baseline) was not. The number of eyes being positive at the InflammaDry test at 30 days (10 eyes, 62.5 %) was significantly lower than those at baseline (all 16 eyes, as per inclusion criteria; p=0.01). The mean OSDI score after thirty days of treatment (44.00±12.00; range 21-56) was lower than that at baseline (47.30±11.00; mean variation, -3.30±6.60 units) but the difference was not statistically significant. The Meibomian gland score was not significantly different (2.80±1.00 at both time points). As far as symptoms were concerned, the conjunctival hyperemia score after 30 days was 1.18±0.75, while that at baseline was 1.64±1.12, the difference being not statistically significant (p = 0.07), but suggestive of a trend implying the tear substitute may improve this symptom. Among self-reported symptoms, burning was significantly lower (1.64±0.81 vs. 2.55±0.69, p = 0.02). Some of the other subjective symptom scores showed a similar, even if not statistically significant, trend toward improvement **(Table 1)**.

No patient experienced any adverse effects. For sake of completeness, results at the contralateral eyes treated with the alternative tear substitute were as follows: the difference in TF- BUT was not statistically significant (4.82±3.10 seconds after 30 days vs. 4.73±3.20 seconds at baseline, p=0.80); the difference in the Oxford score (2.00±1.00 at 30 days vs. 2.09±1.04 at baseline) as well as that in the van Bijsterveld score for Lissamine green staining score (1.00±1.00 at 30 days vs. 1.90±1.00 at baseline) and that in the OSDI score (27.90±13.40, range 19-52 at 30 days vs. 26.60±8.90, range 16-38; mean variation, +1.30±8.30 units) were not statistically significant (p>0.05 in all cases). At 30 days, the eyesthat were positive at the InflammaDry test were 14 (87.5 %) versus 16 at baseline (100%, as per inclusion criteria), the difference being not statistically significant. The Meibomian gland score was not significantly different (3.60±0.50 vs. 3.40 ±0.80, p>0.05). Conjunctival hyperemia at 30 days was not significantly different from that at baseline (1.73±1.19 vs. 1.64±0.67, p>0.05). Even the scores concerning the other symptoms of interest did not change significantly (burning,1.36±0.67 vs 1.82±1.08; photophobia, 2.91±1.30 vs. 3.45±0.93; foreign body sensation, 1.55±0.69 vs. 1.91±1.04; itchy eyes, 1.45±0.82 vs. 1.27±1.27; tearing, 2.27±0.79 vs 2.55±1.04; p>0.05 in all cases). No patient complained concerning any adverse effects.

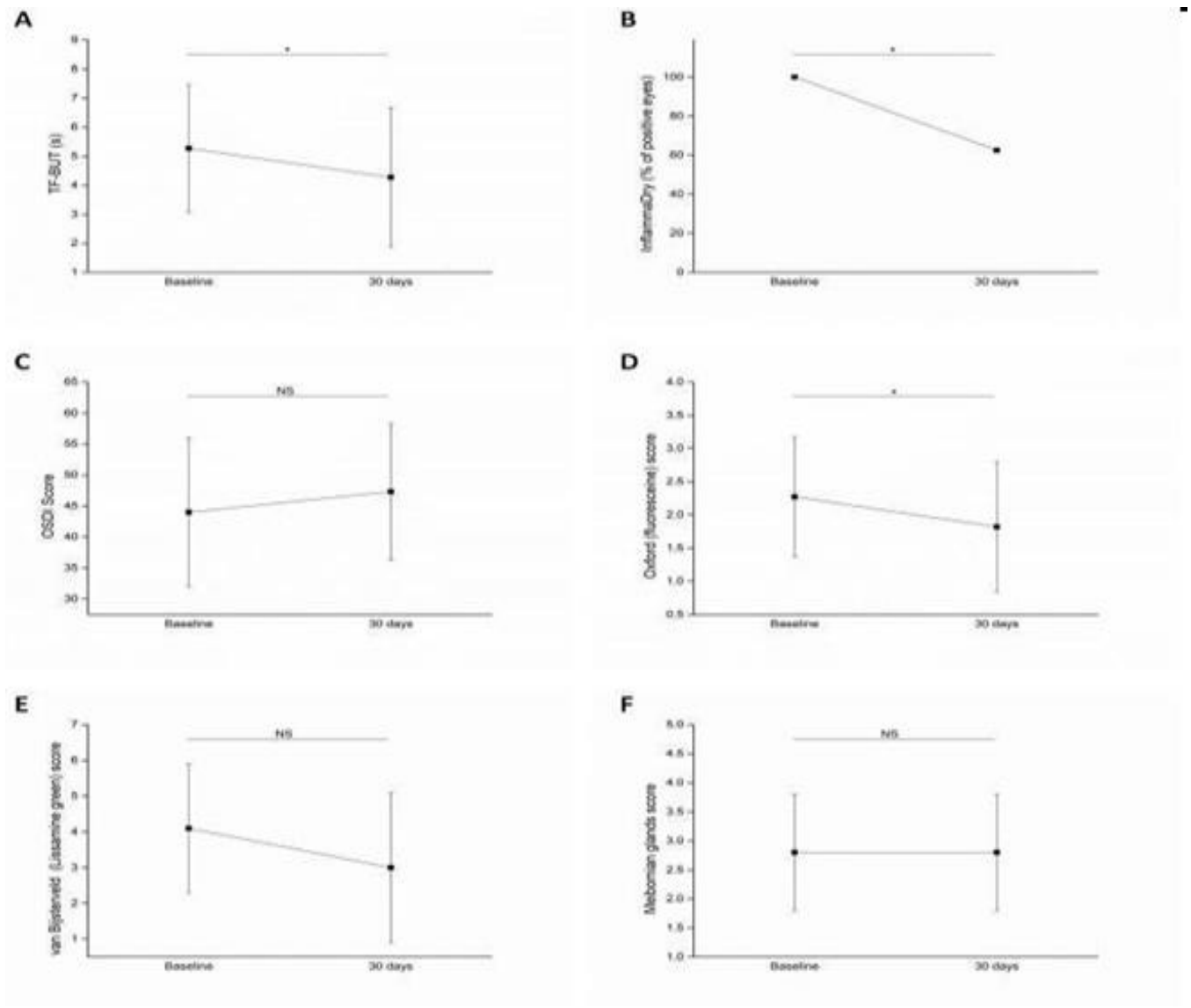

Figure 1. Plots showing the variation of the main study endpoints after DED-affected eyes were treated using the investigational device. *marks significance; NS, not significant. A) the TF-BUT showed a significant decrease; B) the fraction of patients positive for a pathologic MMP-9 level also significantly decreased; C) the OSDI score increased, but the difference was not statistically significant; D) the Oxford fluoresceine staining score decreased significantly; the E) van Bijsterveld Lissamine staining score decreased, but the difference was not statistically significant. The F) Meibomian gland score did not change.

Table 1. Signs and symptoms of DED measured on the day of enrollment (baseline) and 30 days after treatment using the investigational device.

	Baseline	**30 Days**	***p-value***
Conjunctival hyperemia	1.64±1.12	1.18±0.75	*0.07*
Burning	2.55±0.69	1.64±0.81	*0.02**
Photophobia	1.82±1.40	1.55±1.13	*0.19*
Foreign body sensation	1.82±1.33	1.91±1.58	*1.00*
Itchy eyes	1.91±0.94	1.55±0.52	*0.23*
Tearing	1.73±0.90	2.18±1.17	*0.44*

* marks significance. Conjunctival hyperemia was assessed by the clinician; all other symptoms were self-assessed by patients.

Discussion

Results of the present study suggest that Lacrisek Ofta Plus is effective in managing DED symptoms when applied daily over 30 days as per its manufacturer instructions. Most primary study endpoints, namely the mean TF-BUT time, the number of eyes positive to the InflammaDry test, and the fluoresceine Oxford score concerning ocular surface staining pattern were, in fact, significantly

improved after the treatment period compared to baseline. The OSDI score increased, even if the difference was not statistically significant (possibly because of the small sample size). These positive outcomes may be ascribed to the tear substitute composition. Lipid-based and liposome-based tear substitutes are known to be beneficial because they mimic – better than water-based eye drops – the aqueous and lipid layers of the tear film **[16]**. Results of the present study are indeed consistent with previously published evidence concerning using lipid- based and liposome-based substitutes to manage DED **[17- 20,31-36]**. Results of the present study are also consistent with clinical observations concerning lipid-based tear substitutes containing amino acids. Indeed, similar beneficial effects were reported in a prospective, double-masked, controlled, clinical study comparing the effects of two tear substitutes based on sodium hyaluronate (SH) alone or SH enriched with amino acids (L-glycine, L-proline, L-lysine hydrochloride, L-leucine) in patients with tear film disorders. After 1 month of treatment, a statistically significant improvement of TF- BUT was observed in the aminoacid-enriched-SH treated group compared to baseline value **[37]**; eyes treated with eye drops containing amino acids had better corneal staining, and observation at the confocal microscope showed less hyper-reflecting cells (a sign of metabolic damage) in the epithelial corneal layer compared to those receiving hyaluronic acid alone. Results of the present study, as well as those by Aragona et al. **[37]**, are consistent with the supposed beneficial effect ascribed to exogenous supplementation of amino acids (including L-proline, L-glycine, L-lysine, and L-leucine) through tear substitutes **[23]**, given the fact that their concentration changes in patients affected by DED **[38]** as well as in other ocular pathologies **[38,39]**. Such consistency between the beneficial effect of exogenous amino acid supplementation through tear substitutes in DED patients and the variation of the amino acid level in the pathologic eye represents one topic at the forefront of current research **[23,38]** and should be the subject of further investigations. As in the present study, positive clinical results have also been reported in using eye drops containing vitamin A or E to manage DED **[24-26]**. As far as the investigational device that was preliminary assessed in the present study is concerned, as well as for the different tear substitutes currently marketed to manage DED, it is not possible to tell which of the formulation components may have been more relevant in determining efficacy, let alone to identify if they have had any synergistic effect. Indeed, despite the widespread of DED, only a few randomized clinical trials (RCTs) have compared the efficacy of different tear substitutes **[40]**. In 2016, Pucker et al. reviewed 43 RCTs assessing the efficacy of several tear substitutes in DED and performed a meta-analysis of those comparing head-to-head different tear substitutes; the analysis showed most of the tear substitutes tested were equally effective, concluding that at present the literature indicates uncertainty as to which tear substitute works best **[41]**. Indeed, studies aimed to collect evidence-based guidance for managing DED through artificial tear supplements are only very recent **[42]**. Incidentally, data of the present study seem to suggest the alternative tear substitute was not as effective as the investigational one. Yet, data were not subjected to statistical analysis, which would have been out of the scope of this investigation and meaningless given its small sample size; the small sample size itself might have hidden significant results that could have instead been observed by assessing a greater number of subjects. This again confirms that thorough, well-designed appropriate clinical studies should be performed to better distinguish if any currently marketed tear substitute performs better than others. Limitations of the present study arise from it being altogether preliminary in nature and include the reduced sample size, the lack of a control group, and the limited follow-up. Studies with a greater number of subjects would allow choosing appropriate control groups, either including healthy subjects, or subjects to be treated with a comparator. Longer follow-up times would be required to assess longer-term efficacy and safety, including the development of habituation. Appropriate comparative studies would also allow to fine- tune the composition of the eyedrop formulations to specific subgroups of patients, suffering from DED with varying severity.

Conclusions

Within the limitations of the present study, the investigational device, Lacrisek Ofta Plus, seems to be safe and effective in managing symptoms of dry eye disease, consistently with the beneficial effects ascribed to its liposome-based formulation, containing L-proline, L-glycine, L-lysine hydrochloride, and L-leucine as well as vitamin A and E. In turn, the results of the present study provide additional evidence concerning the effectiveness and safety of these components when used to treat DED. These preliminary results should be confirmed by studies involving a greater number of subjects and a longer follow-up. The effectiveness and safety of Lacrisek Ofta Plus should also be compared to those of other currently marketed lipid/liposome-based tear substitutes by carrying out appropriately designed comparative clinical investigations.

Author's contribution: The authors have contributed equally to this work.

Author Disclosure Statement: No competing financial interests exist.

Funding sources: No funding was received for this study.

References

1. Yazdani M, Elgstøen KBP, Rootwelt H, Shahdadfar A, Utheim ØA, et al. (2019) Tear Metabolomics in Dry Eye Disease: A Review. Int J Mol Sci. 20(15): 3755.
2. Şimşek C, Doğru M, Kojima T, Tsubota K (2018) Current management and treatment of dry eye disease. Turkish J Ophthalmol. 48(6): 309-313.
3. Sharma A, Hindman HB (2014) Aging: A Predisposition to Dry Eyes. J Ophthalmol. 2014: 1-8.
4. Aggarwal S, Galor A (2018) What's new in dry eye disease diagnosis? Current advances and challenges. F1000Res. 7: F1000 Faculty Rev-1952.
5. Craig JP, Nichols KK, Akpek EK, Caffery B, Dua HS, et al. (2017) TFOS DEWS II Definition and Classification Report. Ocul Surf. 15(3): 276-283.
6. Jones L, Downie LE, Korb D, Benitez-Del-Castillo JM, Dana R, et al. (2017) TFOS DEWS II Management and Therapy Report. Ocul Surf. 15(3): 575-628.
7. Miljanović B, Dana R, Sullivan DA, Schaumberg DA (2007) Impact of Dry Eye Syndrome on Vision-Related Quality of Life. Am J Ophthalmol. 143(3): 409-415.
8. Li M, Gong L, Sun X, Chapin WJ (2011) Anxiety anddepression in patients with dry eye syndrome. Curr Eye Res. 36(1): 1-7.
9. Lemp MA, Crews LA, Bron AJ, Foulks GN, Sullivan BD (2012) Distribution of aqueous-deficient and evaporative dry eye in a clinic-based patient cohort: a retrospective study. Cornea. 31(5): 472-478.
10. Seitzman G, Lietman T (2018) Dry eye. N Engl J Med. 379(11): e19.
11. Schiffman RM, Christianson MD, Jacobsen G, Hirsch JD, Reis BL (2000) Reliability and validity of the Ocular Surface Disease Index. Arch Ophthalmol. 118(5): 615-621.
12. Wolffsohn JS, Arita R, Chalmers R, Djalilian A, Dogru M, et al. (2017) TFOS DEWS II Diagnostic Methodology report. Ocul Surf. 15(3): 539-574.
13. Leonardi A, Brun P, Abatangelo G, Plebani M, Secchi AG (2003) Tear levels and activity of matrix metalloproteinase (MMP)-1 and MMP-9 in vernal keratoconjunctivitis. Investig Ophthalmol Vis Sci. 44(7): 3052-3058.
14. Messmer EM, von Lindenfels V, Garbe A, Kampik A (2016) Matrix Metalloproteinase 9 Testing in Dry Eye Disease Using a Commercially Available Point-of-Care Immunoassay. Ophthalmology. 123(11): 2300-2308.
15. Acera A, Rocha G, Vecino E, Lema I, Durán JA (2008) Inflammatory markers in the tears of patients with ocular surface disease. Ophthalmic Res. 40(6): 315-321.
16. Garrigue JS, Amrane M, Faure MO, Holopainen JM., Tong L (2017) Relevance of Lipid-Based Products in the Management of Dry Eye Disease. J Ocul Pharmacol Ther. 33(9): 647-661.
17. Craig JP, Purslow C, Murphy PJ, Wolffsohn JS (2010) Effect of a liposomal spray on the pre-ocular tear film. Cont Lens Anterior Eye. 33(2): 83-7.
18. McGinnigle S, Eperjesi F, Naroo SA (2014) A preliminary investigation into the effects of ocular lubricants on higher order aberrations in normal and dry eye subjects. Cont Lens Anterior Eye. 37(2): 106-10.
19. Nosch DS, Joos RE, Job M (2020) Prospective randomized study to evaluate the efficacy and tolerability of Ectoin containing Eye Spray (EES09) and comparison to the liposomal Eye Spray Tears Again (TA) in the treatment of dry eye disease. Cont Lens Anterior Eye. 27: S1367-0484(20)30076-X.
20. Sánchez-González JM, De-Hita-Cantalejo C, Sánchez-González MC (2020) Crosslinked hyaluronic acid with liposomes and crocin for management symptoms of dry eye disease caused by moderate meibomian gland dysfunction.Int J Ophthalmol. 13(9): 1368-1373.
21. López-Cano JJ, González-Cela-Casamayor MA, Andrés-Guerrero V, Herrero-Vanrell R, Molina-Martínez IT (2021) Liposomes as vehicles for topical ophthalmic drug delivery and ocular surface protection. Expert Opin Drug Deliv. 22:1-29.
22. Wegener AR, Meyer LM, Schönfeld CL (2015) Effect of Viscous Agents on Corneal Density in Dry Eye Disease. J Ocul Pharmacol Ther. 31(8): 504-508.
23. Rusciano D, Roszkowska AM, Gagliano C, Pezzino S (2016) Free amino acids: an innovative treatment for ocular surface disease. Eur J Pharmacol. 787: 9-19.
24. Kim EC, Choi JS, Joo CK (2009) A Comparison of Vitamin A and Cyclosporine A 0.05% Eye Drops for Treatment of Dry Eye Syndrome. Am J Ophthalmol. 147(2): 206-213.e3.
25. Toshida H, Funaki T, Ono K, Tabuchi N, Watanabe S, et al. (2017) Efficacy and safety of retinol palmitate ophthalmic solution in the treatment of dry eye: a Japanese Phase II clinical trial. Drug Des Devel Ther. 11: 1871-1879.
26. Serrano-Morales JM, De-Hita-Cantalejo C, Sánchez-González MC, Bautista-Llamas MJ, Sánchez-González JM (2020) Efficacy of 0.1% crosslinked hyaluronic acid, coenzyme Q10 and vitamin E in the management of dry eye disease in menopause patients receiving antidepressants. Eur J Ophthalmol. Nov 5:1120672120972026.
27. Bron AJ, Evans VE, Smith JA (2003) Grading of corneal and conjunctival staining in the context of other dry eye tests. Cornea. 22(7): 640-50.
28. van Bijsterveld OP (1969) Diagnostic tests in the Sicca syndrome. Arch Ophthalmol. 82(1): 10-4
29. Sambursky R Davitt, WF. 3rd, Friedberg M, Tauber S (2014) Prospective, multicenter, clinical evaluation of point-of-care matrix metalloproteinase-9 test for confirming dry eye disease.

Cornea. 33(8): 812-818.

30. Sambursky R, Davitt WF 3rd, Latkany R, Tauber S, Starr C, et al. (2013) Sensitivity and specificity of a point-of-care matrix metalloproteinase 9 immunoassay for diagnosing inflammation related to dry eye. JAMA Ophthalmol. 131(1): 24-8.
31. Kaercher T, Thelen U, Brief G, Morgan-Warren RJ, Leaback R (2014) A prospective, multicenter, noninterventional study of Optive Plus in the treatment of patients with dry eye: the prolipid study. Clin Ophthalmol. 8: 1147-55.
32. Martin E, Oliver KM, Pearce EI, Tomlinson A, Simmons P, et al. (2018) Effect of tear supplements on signs, symptoms and inflammatory markers in dry eye. Cytokine. 105: 37-44.
33. Baudouin C, Galarreta DJ, Mrukwa-Kominek E, Böhringer D, Maurino V, et al. (2017) Clinical evaluation of an oil-based lubricant eyedrop in dry eye patients with lipid deficiency. Eur J Ophthalmol. 27(2): 122-128.
34. Simmons PA, Carlisle-Wilcox C, Chen R, Liu H, Vehige JG (2015) Efficacy, safety, and acceptability of a lipid-based artificial tear formulation: a randomized, controlled, multicenter clinical trial. Clin Ther. 37(4): 858-68.
35. McCann LC, Tomlinson A, Pearce EI, Papa V (2012) Effectiveness of artificial tears in the management of evaporative dry eye. Cornea. 31(1): 1-5.
36. Fogagnolo P, Ottobelli L, Digiuni M, Rossetti L. Short – term efficacy of two lipidic eyedrops in the treatment of evaporative dry eye. Italian Review of Ophthalmology. 2016;2(2): 97-105.
37. Aragona P, Rania L, Roszkowska AM, Spinella R, Postorino E, et al. (2013) Effects of amino acids enriched tears substitutes on the cornea of patients with dysfunctional tear syndrome. Acta Ophthalmol. 91(6): e437-44.
38. Nakatsukasa M, Sotozono C, Shimbo K, Ono N, Miyano H, etal. (2011) Amino Acid profiles in human tear fluids analyzed by high-performance liquid chromatography and electrospray ionization tandem mass spectrometry. Am J Ophthalmol. 151(5): 799-808.e1
39. Rossi C, Cicalini I, Cufaro MC, Agnifili L, Mastropasqua L, et al. (2019) Multi-Omics Approach for Studying Tears in Treatment-Naive Glaucoma Patients. Int J Mol Sci. 20(16): 4029.
40. Lee S-Y, Tong L. (2012) Lipid-containing lubricants for dry eye: a systematic review. Optom Vis Sci. 89(11): 1654-1661.
41. Pucker AD, Ng SM, Nichols JJ (2016) Over the counter (OTC) artificial tear drops for dry eye syndrome. Cochrane Database Syst Rev. 2: :CD009729.
42. Craig JP, Muntz A, Wang MTM, Luensmann D, Tan J, et al. (2021) Developing evidence-based guidance for the treatment of dry eye disease with artificial tear supplements: A six-month multicentre, double-masked randomised controlled trial. Ocul Surf. 20: 62-69.