Effects of Topical Cyclosporine A Plus Artificial Tears Versus Artificial Tears Treatment on Conjunctival Goblet Cell Density in Dysfunctional Tear Syndrome

Elvan Demiryay, M.D., Volkan Yaylalı, M.D., Ebru Nevin Cetin, M.D., and Cem Yıldırım, M.D.

Objectives: The aim was to compare the effects of topical cyclosporine A and artificial tears combination with artificial tears alone in patients with dysfunctional tear syndrome (DTS).

Methods: Forty-two eyes of 42 patients with DTS were enrolled in the study. The inclusion criteria for the study were Schirmer I (without anesthesia) scores below 10 mm/5 min and tear film break-up time (BUT) below 10 sec. The patients were randomly divided into two groups. The study group (22 patients) underwent 0.05% cyclosporine A treatment twice a day and preservative-free artificial tears for four times a day for 4 months. The control group (20 patients) was administered only preservative-free artificial tears four times a day for 4 months. The BUT, Schirmer test scores, corneal fluorescein staining, conjunctival lissamine green staining, and goblet cell density derived by impression cytology were recorded before and after treatment in each group.

Results: In the study group, all parameters improved statistically significantly after treatment at the 4-month follow-up compared with the pretreatment values ($P<0.001$ for all). In the control group, corneal fluorescein staining ($P<0.001$) and conjunctival lissamine green staining ($P=0.014$) improved, but BUT and Schirmer scores did not change significantly after treatment. At the end of the 4-month follow-up, the study group demonstrated statistically significantly better BUT ($P=0.020$), Schirmer scores ($P=0.002$), goblet cell density ($P=0.006$), corneal fluorescein staining ($P=0.003$), and conjunctival lissamine green staining ($P=0.017$) scores than did the control group.

Conclusions: Topical cyclosporine A and artificial tears treatment significantly increases goblet cell density, decreases the signs of DTS, and improves ocular surface health.

Key Words: Cyclosporine—Dry eye—Dysfunctional tear syndrome—Goblet cell—Impression cytology.

(*Eye & Contact Lens* 2011;37: 312–315)

Dry eye syndrome or ''dysfunctional tear syndrome'' (DTS), as recommended by the Delphi panel,[1] is a multifactorial disease of the tears and ocular surface that causes symptoms of discomfort, visual disturbance, and tear film instability with potential damage to the ocular surface.[2] Up to now, there have been numerous approaches for the management of DTS consisting of avoidance of exacerbating factors, eyelid hygiene, artificial tears and lubricants, punctal plugs, tear stimulation, and anti-inflammatory agents.[3] Topical corticosteroids are effective anti-inflammatory agents, but their side effects limit long-term use.[3] Topical cyclosporine A has been reported to inhibit epithelial apoptosis and cytokine production from the activated T-lymphocytes,[4–6] reduce inflammatory and apoptotic markers,[7] increase goblet cell density, and improve tear film stability.[8,9] The purpose of this study is to compare the effects of topical cyclosporine A and artificial tears combination with artificial tears alone on ocular surface health and tear film stability in patients with DTS.

MATERIALS AND METHODS

A total of 42 eyes of 42 DTS patients were enrolled in this prospective randomized and partially masked study. The inclusion criteria for the study were Schirmer I (without anesthesia) scores below 10 mm/5 min and tear film break-up time (BUT) below 10 sec as defined for mild to severe patients with DTS in the DEWS grading scheme.[10] Exclusion criteria for the study were history of systemic or ocular diseases (including ocular surgery and trauma), use of ophthalmic or systemic medications (including artificial tears), and pregnancy. The study was performed in compliance with the ethical principles of the Declaration of Helsinki and Good Clinical Practice and was also approved by the ethical committee of our institution. All the patients provided written informed consent and underwent full ophthalmic assessment, including measurement of visual acuity and intraocular pressure and anterior and posterior segment evaluation.

The patients were randomly divided into two groups. The study group (22 patients) underwent 0.05% cyclosporine A (Restasis; Allergan, Irvine, CA) treatment twice a day and preservative-free artificial tears (0.3% hydroxypropyl methylcellulose/0.1% dextran 70-Tears Naturale Free; Alcon Lab, TX) four times a day for 4 months for both eyes. The control group (20 patients) was administered only artificial tears four times a day for 4 months for both eyes. The patients were not masked to the therapy regimens. The BUT, Schirmer test scores, corneal fluorescein staining, conjunctival lissamine green staining, and goblet cell density assessed by impression cytology of the right eyes were recorded before and after treatment. The investigators assessing the test scores were masked to the therapy regimens of the patients. At the first month, the patients had a visit to check the compliance and side

From the Department of Ophthalmology, Niğde State Hospital, Niğde, Turkey; and Department of Ophthalmology (V.Y., C.Y., E.N.C.), Faculty of Medicine, Pamukkale University, Denizli, Turkey.

The authors have no funding or conflicts of interest to disclose.

Address correspondence and reprint requests to Volkan Yaylalı M.D., Pamukkale Üniversitesi, Göz Hastalıkları AD, Kınıklı Kampüsü, Kınıklı, Denizli, Turkey; e-mail: volkanyaylali@yahoo.com

Accepted May 19, 2011.

DOI: 10.1097/ICL.0b013e31822563be

effects of the treatment, but BUT, Schirmer, and impression cytology tests were not performed.

The BUT was evaluated by applying a fluorescein strip into the inferior fornix of both eyes after moistening it with saline solution and throwing the excess drops away.[11] An average of three consecutive measures from the last blink to the first sign of irregularity was recorded. Schirmer I test was performed (without anesthesia) by leaving the strips in the lateral fornices for 5 min. Then, the length of the wet paper was recorded as the Schirmer score. Corneal fluorescein staining and conjunctival lissamine green staining were graded from 0 to 5 according to the Oxford grading scheme.[11]

Impression cytology samples were taken after anesthetizing the eye with topical 0.5% proparacaine hydrochloride. Cellulose acetate filters (Sartorius, 11107–50–N, Göttingen, Germany) with pore sizes of 0.022 to 0.025 μm were cut into 2-mm×3-mm pieces and applied to temporal interpalpebral conjunctiva by pressing the blunt tips of the application forceps for 3 to 5 sec. The filter was grasped gently with the forceps and placed in the fixative containing 70% ethanol, 37% formaldehyde, and glacial acetic acid mixture in the ratio of 20:1:1 and kept in +4°C. The goblet cells on cellulose acetate filters were stained with periodic acid–Schiff—hemalum. Goblet cells were counted in 5 representative microscopic fields (500×500 μm) per membrane with a ×10 magnification (light microscope), and the mean goblet cell count was calculated at each visit.

Statistical Methods and Data Analysis

Wilcoxon and Mann–Whitney *U* tests (two-tailed alpha) were used as statistical methods. $P<0.05$ was considered statistically significant. SPSS v.11 was used for statistical analysis.

RESULTS

Forty-two patients completed the study. A total of 40 patients were female (95.2%) and 2 were male (4.8%). The mean age (and range) of the study population was 45.5±13.2 years (17–66 years) (Table 1).

Tear Film Break-Up Time and Schirmer Test

Before treatment, there was no significant difference in BUT or Schirmer test scores between the two groups ($P=0.592$, $P=0.970$, respectively). After 4 months of treatment, the differences in BUT and Schirmer scores between the study and the control groups were statistically significant, with better scores in the study group ($P=0.020$, $P=0.002$, respectively) (Figs. 1 and 2; Mann–Whitney *U*).

Corneal and Conjunctival Staining

The change in corneal fluorescein staining scores before and after treatment was significant in the study group ($P<0.001$). Before treatment, there was no significant difference in corneal fluorescein staining scores between the study and the control groups ($P=0.643$). After 4 months of treatment, the difference was significant with lower staining scores in the study group ($P=0.003$) (Fig. 3).

The change in conjunctival lissamine green staining scores before and after treatment was significant in the study group ($P<0.001$). Before treatment, there was no significant difference in corneal fluorescein staining scores between the study and the control groups ($P=0.348$). After 4 months of treatment, the difference was significant with lower staining scores in the study group ($P=0.017$) (Fig. 4; Mann–Whitney *U*).

TABLE 1. *Demographic Data of the Patients*

	Study Group (Artificial Tears+Cyclosporine) n = 22	Control Group (Artificial Tears Alone) n=20	Mann–Whitney *U*
Mean age	46.59±12.28	44.3±14.36	$P>0.05$
Gender			$P>0.05$
Male	2 (9%)	0	
Female	20 (90.9%)	20 (100%)	

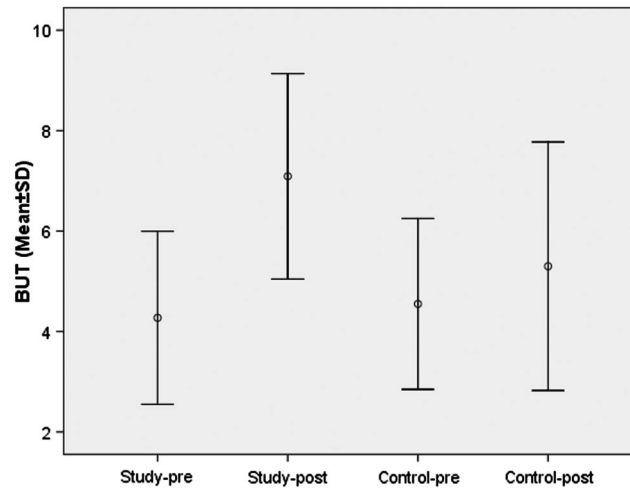

FIG. 1. Mean±SD tear film break-up time (BUT) values before and after treatment in the study group and the control group. Before treatment, there was no significant difference between the two groups ($P=0.592$). After treatment, BUT significantly improved in the study group versus that in the control group ($P=0.020$).

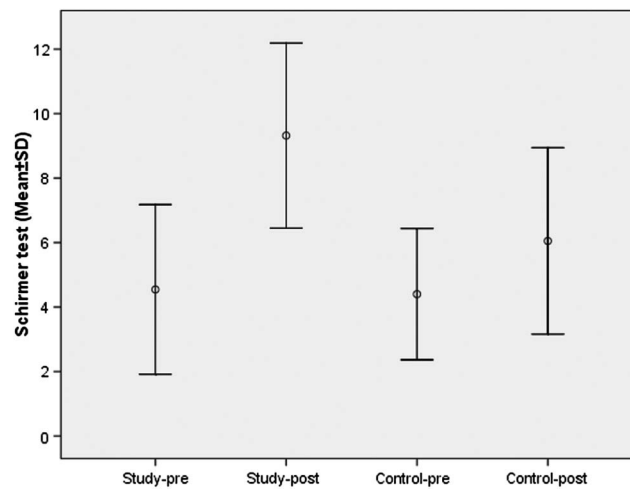

FIG. 2. Mean±SD Schirmer scores before and after treatment in the study group and the control group. Before treatment, there was no significant difference between the two groups ($P=0.970$). After treatment, Schirmer scores significantly improved in the study group versus that in the control group ($P=0.002$).

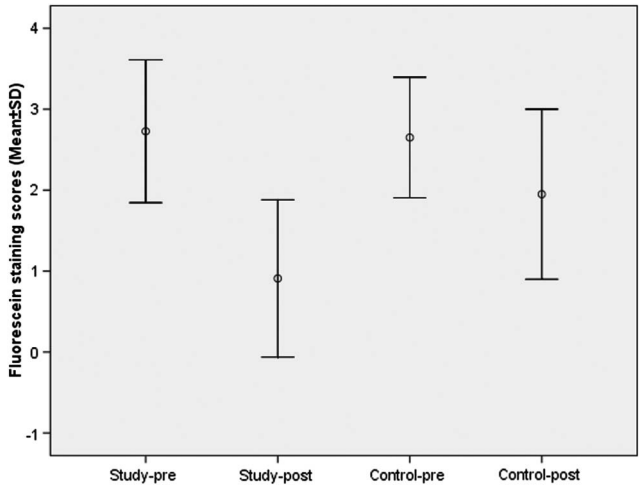

FIG. 3. Mean±SD corneal fluorescein staining scores before and after treatment in the study group and the control group. Before treatment, there was no significant difference between the groups (P=0.643). After treatment, staining scores significantly improved in the study group versus that in the control group (P=0.003).

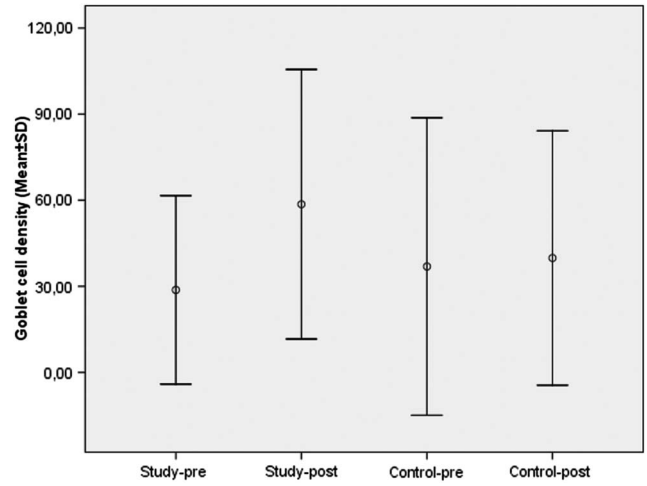

FIG. 5. Mean±SD conjunctival Goblet cell density before and after treatment in the study group and the control group. Before treatment, there was no significant difference between the two groups (P=0.332). After treatment, goblet cell density significantly improved in the study group versus that in the control group (P=0.006).

Goblet Cell Density

There was no significant difference in the goblet cell density between the two groups before treatment (P=0.332). After 4 months of treatment, the difference between the study and the control group was statistically significant (P=0.006). The goblet cell density increased in both the study and the control groups after treatment at the 4-month follow-up, but the difference between pretreatment and posttreatment values was statistically significant only in the study group ($P<0.001$) (Fig. 5).

No ocular or systemic side effects were observed in any of the patients during the study.

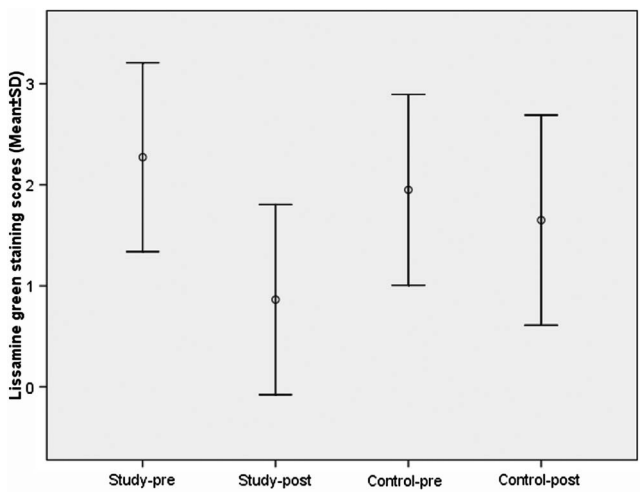

FIG. 4. Mean±SD conjunctival lissamine green staining scores before and after treatment in the study group and the control group. Before treatment, there was no significant difference between the groups (P=0.348). After treatment, staining scores significantly improved in the study group versus that in the control group (P=0.017).

DISCUSSION

T-lymphocyte infiltration of the conjunctiva has been observed in patients with DTS with or without Sjögren syndrome.[12] The effects of cyclosporine including the inhibition of epithelial apoptosis and cytokine production from T lymphocytes seem to break the inflammatory cascade, which plays a major role in the pathogenesis of DTS.[4,5,13] Strong et al.[5] reported reduced conjunctival epithelial apoptosis and protection of goblet cells by topical cyclosporine A treatment in a murine model of keratoconjunctivitis sicca. Mucine, which is secreted by goblet cells, serves as an interface between hydrophobic corneal epithelium and aqueous tear fluid. Increase in the goblet cell density renders increased mucine production and thus better stability of the tear film.[8,14]

The decrease in conjunctival goblet cell density is the first sign of ocular surface disease.[15] Therefore, it has become one of the most important parameters in assessing the effect of treatment in DTS. Moon et al.[8] compared the short-term effects (6–8 weeks) of topical 0.05% cyclosporine A versus a mixture of 0.08% chondroitin sulfate and 0.06% sodium hyaluronate in DTS. After treatment, they found out that the ocular surface improved in both groups, but tear film stability and goblet cell density increased more effectively in the cyclosporine A treatment group. Kunert et al.[16] also reported an increase in the number of goblet cells in patients with non-Sjögren syndrome associated keratoconjunctivitis sicca and Sjögren syndrome associated keratoconjunctivitis sicca on treatment with topical cyclosporine A for 6 months.

In another study, Pflugfelder et al.[9] evaluated the effects of sequential artificial tears and topical cyclosporine emulsion therapy on goblet cell density in DTS. The increase in goblet cell density was significant after 3 months of cyclosporine A emulsion therapy but not after the administration of artificial tears. Albietz and Bruce.[15] showed that conjunctival inflammation and goblet cell numbers were not significantly changed by nonpreserved artificial tears but got even worse on using preserved ones. In our study, there

was an increase in the number of goblet cells in the study and the control groups after treatment, but the difference was statistically significant in only the study group, in agreement with the findings of the previous studies.[9,15]

We observed a significant difference in Schirmer scores, BUT, fluorescein, and lissamine green staining between the study group and the control group after 4 months of treatment. The differences in fluorescein and lissamine green staining between pretreatment and posttreatment values were also significant in both groups. Sall et al.[17] evaluated the effect of cyclosporine A and artificial tears in patients with DTS. The first group underwent topical 0.05% cyclosporine A and 0.001% polyquad combination treatment, the second group underwent topical 0.05% cyclosporine A and 0.5% carboxymethylcellulose sodium combination treatment, and the third group underwent 0.001% polyquad treatment. The first group showed the greatest improvement in fluorescein corneal staining from baseline. There were small and insignificant changes in Schirmer scores in all the groups. In the study of Moon et al.,[8] there was significant prolongation of BUT and improvement in Schirmer scores in both groups (0.05% cyclosporine A vs. 0.08% chondroitin sulfate and 0.06% sodium hyaluronate), which was more prominent in the first group. In another study, Kim et al.[18] reported a significant improvement in BUT, Schirmer scores, and impression cytology findings in patients with DTS, with topical cyclosporine A and artificial tears versus with artificial tears alone.

In the Phase 3 study, Schirmer scores with anesthesia showed a significant difference between the 0.05% CsA and vehicle groups.[14] Schirmer scores without anesthesia were not significantly different between the CsA and vehicle groups, but both the groups showed significant differences compared with baseline values. Reflexive tearing significantly improved in the CsA group and in the vehicle group compared with baseline values. We also found a significant difference in the Schirmer scores between the pretreatment and posttreatment values in the study group. Our results differ from those of the Phase 3 study when the study and the control groups were compared. There are possible explanations for this difference: The first is that the vehicle may be more effective than artificial tears (the pretreatment and posttreatment schirmer scores in the vehicle group were also significant in the Phase 3 study). The second is that our study group may have a synergistic effect because it does not only consist of CsA but also CsA+vehicle+artificial tears and may have a stronger effect than does CsA alone. The third explanation is that the duration of the studies may cause the differences. In the Phase 3 study, the follow-up time is 6 months, which is longer than that of our study and of the previous studies with significant Schirmer improvements,[8,18] so the effect on Schirmer scores in our study may be a short-term effect.

In our study group, we did not observe ocular side effects such as burning, stinging, and foreign body sensation, which were reported in the Phase 3 study. This may be the effect of artificial tears, which were a part of the treatment in our study group.

There are several limitations for our study: The first limitation is its partially masked design. The investigator assessing the tests was masked to the therapy regimen, but the patients were not. Additionally, any effects seen in the study group are not just related to cyclosporine but also to the vehicle of cyclosporine and artificial tears. A synergistic effect is also possible. Another limitation is the lack of information about the symptoms. Our study provides data about the effect of CsA and artificial tears on objective signs of dysfunctional tears syndrome, especially on the goblet cell density. The final limitation is the power of the study. Larger groups are needed for achieving higher statistical power.

Consequently, in this study, topical cyclosporine A with artificial tears is more effective in treating signs of DTS than artificial tears alone.

ACKNOWLEDGMENTS

In this study, for statistical analysis, the authors consulted Mehmet Zencir, Associate professor, Department of Public Health, Faculty of Medicine, Pamukkale University, Denizli, Turkey.

REFERENCES

1. Behrens A, Doyle JJ, Stern L, et al. Dysfunctional tear syndrome study group. Dysfunctional tear syndrome: A Delphi approach to treatment recommendations. *Cornea* 2006;25:900–907.
2. The definition and classification of dry eye disease: Report of the definition and classification subcommittee of the international dry eye workShop (2007) [Review]. *Ocul Surf* 2007;5:75–92.
3. Lemp MA. Management of dry eye disease [Review]. *Am J Manag Care* 2008;14:S88–S101.
4. Matsuda S, Koyasu S. Mechanisms of action of cyclosporine [Review]. *Immunopharmacology* 2000;47:119–125.
5. Strong B, Farley W, Stern ME, et al. Topical cyclosporine inhibits conjunctival epithelial apoptosis in experimental murine keratoconjunctivitis sicca. *Cornea* 2005;24:80–85.
6. Kunert KS, Tisdale AS, Stern ME, et al. Analysis of topical cyclosporine treatment of patients with dry eye syndrome. *Arch Ophthalmol* 2000;118: 1489–1496.
7. Brignole F, Pisella PJ, De Saint Jean M, et al. Flow cytometric analysis of inflammatory markers in KCS: 6-month treatment with topical cyclosporin A. *Invest Ophthalmol Vis Sci* 2001;42:90–95.
8. Moon JW, Lee HJ, Shin KC, et al. Short term effects of topical cyclosporine and viscoelastic on the ocular surface in patients with dry eye. *Korean J Ophthalmol* 2007;21:189–194.
9. Pflugfelder SC, De Paiva CS, Villarreal AL, et al. Effects of sequential artificial tear and cyclosporine emulsion therapy on conjunctival goblet cell density and transforming growth factor-beta2 production. *Cornea* 2008; 27:64–69.
10. Management and therapy of dry eye disease: Report of the management and therapy subcommittee of the international dry eye workshop (2007). *Ocul Surf* 2007;5:163–178.
11. Bron AJ, Evans VE, Smith JA. Grading of corneal and conjunctival staining in the context of other dry eye tests [Review]. *Cornea* 2003;22:640–650.
12. Stern ME, Gao J, Schwalb TA, et al. Conjunctival T-cell subpopulations in Sjögren's and non-Sjögren's patients with dry eye. *Invest Ophthalmol Vis Sci* 2002;43:2609–2614.
13. Hyon JY, Lee YJ, Yun PY. Management of ocular surface inflammation in Sjogren syndrome. *Cornea* 2007;26:13–15.
14. Sall K, Stevenson OD, Mundorf TK, et al. Two multicenter, randomized studies of the efficacy and safety of cyclosporine ophthalmic emulsion in moderate to severe dry eye disease. CsA Phase 3 Study Group. *Ophthalmology* 2000;107:631–639.
15. Albietz JM, Bruce AS. The conjunctival epithelium in dry eye subtypes: Effect of preserved and non-preserved topical treatments. *Curr Eye Res* 2001; 22:8–18.
16. Kunert KS, Tisdale AS, Gipson IK. Goblet cell numbers and epithelial proliferation in the conjunctiva of patients with dry eye syndrome treated with cyclosporine. *Arch Ophthalmol* 2002;120:330–337.
17. Sall KN, Cohen SM, Christensen MT, et al. An evaluation of the efficacy of a cyclosporine-based dry eye therapy when used with marketed artificial tears as supportive therapy in dry eye. *Eye Contact Lens* 2006;32:21–26.
18. Kim EC, Choi JS, Joo CK. A comparison of vitamin a and cyclosporine a 0.05% eye drops for treatment of dry eye syndrome. *Am J Ophthalmol* 2009;147:206–213.

