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Exploration of Efficacy and Mechanism of 0.05% Cyclosporine Eye Drops (II) Monotherapy in Allergic Conjunctivitis-associated Dry Eye

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Article

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Abstract

Purpose

To explore the efficacy and relevant mechanism of 0.05% cyclosporine A (CsA) eye drops (II) monotherapy in patients with allergic conjunctivitis-associated dry eye (ACDE).

Methods

Prospective, randomized, controlled study. Fifty-three patients with mild-to-moderate ACDE were randomly assigned to two groups. The CsA group received 0.05% CsA eye drops (II) monotherapy four times daily. The control group received 0.1% olopatadine twice daily combined with 0.1% preservative-free artificial tears four times daily. Clinical symptoms and signs, tear total IgE, and lymphotoxin-α (LT-α) concentrations were assessed at pre- and post-treatment days 7, 30, and 60. And we further measured six tear inflammatory cytokines levels using a microsphere-based immunoassay.

Results

The CsA group showed significant improvement in symptoms (Ocular Surface Disease Index and itching scores) and signs (conjunctival hyperemia, conjunctival edema, conjunctival papillae, tear break-up time (TBUT), corneal fluorescent staining, and goblet cell density) at each follow-up period compared to pre-treatment (all P<0.050). And its improvement in itching scores (P_{7th}<0.001, P_{30th}=0.039, and P_{60th}=0.031) and TBUT (P_{7th}=0.009, P_{30th}=0.003, and P_{60th}=0.005) was more significant than the control group at all follow-up periods. The tear total IgE, interleukin (IL)-5, IL-6, periostin, eotaxin-3, and MMP-9 levels significantly decreased in the CsA group at day 60 after treatment (all P<0.050). And the changed values in tear total IgE were positively correlated with the change in itching scores.

Conclusions

0.05% CsA eye drops (II) monotherapy can rapidly improve the symptoms and signs, especially in ocular itching and TBUT, in patients with ACDE. And its efficacy is superior to 0.1% olopatadine combined with artificial tears. Moreover, CsA downregulates the expression levels of tear inflammatory cytokines, including tear total IgE, IL-5, IL-6, periostin, eotaxin-3, and MMP-9. Among that, the reduction in tear total IgE levels may reflect the improvement of ocular itching.

Introduction

Dry eye (DE) is a multifactorial ocular surface disease prone to recurrent exacerbations, characterized by tear film instability, elevated tear osmolarity, ocular surface inflammation, and neurosensory abnormalities.[1] Allergic conjunctivitis (AC) is a group of immune ocular surface diseases in which hypersensitivity reactions are triggered by allergen stimulation. AC is one of the high-risk factors for provoking or exacerbating DE, the two are concomitant and causal to each other.[2,3] Studies have shown

that the prevalence of DE in patients with AC is 31.3% and even up to 47.2% in a recent meta-analysis.[4,5] In southwest China, the prevalence of DE among children with AC has reached 97.5%.[6] Previously, the major treatment options required a combination of multiple medications. For instance, dual-acting antihistamine/mast-cell stabilizers, topical steroids, or NSAIDs combined with artificial tears.[7,8] However, long-term use of steroids may cause high intraocular pressure, cataracts, and other adverse effects.[9] Meanwhile, such combinations will increase the burden of ocular drug administration and even raise the risk of ocular surface drug toxicity, reduce patient compliance, and affect treatment outcomes.[10,11] Thus, there is an urgent need to explore more effective, simpler, and safer treatment alternatives.

Some scholars suggested that topical cyclosporine A (CsA) could be taken to treat DE or AC, reduce the use of artificial tears and lessen reliance on steroids.[7] CsA is an effective immunomodulator that mainly affects T lymphocytes by inhibiting the activation and decreasing multiple inflammatory cytokines. It contributes to a robust anti-inflammatory effect, blocks the vicious cycle of inflammation, promotes mucin and tear secretion, and is therefore used to treat DE.[12] In addition, CsA can reduce the allergic response by preventing mast cells (MCs) and basophils degranulation, and simultaneously suppressing eosinophil recruitment.[7] Given this, it might apply to treating type I hypersensitivity-mediated AC. Clinically the most common AC types are mild-to-moderate seasonal AC (SAC) and perennial AC (PAC), with SAC accounting for approximately 90% of total AC in the US.[13] However, most studies focus on CsA in treating DE or severe AC, mainly vernal keratoconjunctivitis (VKC) and atopic keratoconjunctivitis (AKC). There is a lack of clinical studies of CsA in patients with mild-to-moderate AC-associated DE (ACDE).

ACDE refers to patients with AC combined with DE, including DE caused by SAC, PAC, VKC, AKC and giant papillary conjunctivitis (GPC). Traditionally, SAC and PAC are caused predominantly by IgE-mediated type I hypersensitivity responses.[6,7] Studies have revealed that total IgE in tears is a reliable predictor of the severity and prognosis of SAC patients.[14] Moreover, lymphotoxin-alpha (LT-α) is a member of the tumor necrosis factor (TNF)-related cytokines family, primarily generated by lymphocytes, which has been considered a diagnostic indicator for DE recently.[15] Apart from these, the primary relevant cytokines of ocular allergy and DE can be used as indicators of disease severity, such as IL-4, IL-5, IL-6, and TNF-α.[16] The 0.05% CsA eye drops (II) are the first approved formulation of cyclosporine ophthalmic nanoparticles for DE patients in China, with advantages in long-term ocular tolerability, preservative-free, and bioavailability.[12,17] However, there is no universal consensus concerning its therapeutic effects in ACDE patients. This study aimed to evaluate the efficacy and explore relevant mechanisms of 0.05% CsA eye drops (II) monotherapy in patients with ACDE.

Methods

• Study design and participants

This was a prospective, randomized, controlled clinical study from July 2021 to November 2022 at the Ophthalmology Clinic of Tianjin Medical University Eye Hospital. All procedures were performed under the Declaration of Helsinki and approved by the Institutional Ethics Committee of the hospital (2020KY-35). Each individual provided written informed consent prior to participating in the trial. The study was registered at http://www.chictr.org.cn (No. ChiCTR2100049498). All participants were binocular, and the eye with severe symptoms was selected. If both eyes were of equal severity, the right eye was selected.

Inclusion criteria were as follows: (1) age 18-70 years; (2) patients who underwent total IgE test in tears and were confirmed by an ophthalmologist to meet the diagnosis of SAC/PAC (according to the *Guidelines for Allergic Conjunctival Diseases 2020*); (3) and patients met the diagnosis of mild-to-moderate dry eye (based on *2017 TFOS DEWS II report classification criteria*). Exclusion criteria included: (1) patients with other types of AC, including AKC, VKC, and GPC; (2) patients who underwent any other infectious inflammation or ocular immunological diseases other than SAC/PAC and DE, such as uveitis, blepharitis, Sjögren's syndrome (SS) and ocular graft-versus-host disease (oGVHD), etc. 3 individuals who had undergone ocular surgery within three months preceding the trial; (4) patients using topical or systemic steroid, anti-allergic or immunosuppressive drugs within one month of inclusion in the study; (5) For tear proteomics analysis, we further excluded subjects with insufficient tear sample size.

After screening a total of 60 patients, 53 patients (18 men and 35 women; age range:18-62 years) with mild-to-moderate ACDE were enrolled and randomly assigned to two groups: CsA group (n=28, 0.05% CsA eye drops (II) four times daily, Sinqi Pharmaceutical, Shenyang, China), Control group (n=25, 0.1% olopatadine hydrochloride twice daily, Huonland, Beijing, China, combined with 0.1% preservative-free sodium hyaluronate artificial tears four times daily, Sinqi Pharmaceutical, Shenyang, China). Subjects were then followed up at baseline and days 7, 30, and 60 after treatment (**Figure 1**).

• Study participant examination

All subjects were evaluated by the same operator. Efficacy observation indicators were subjective symptoms (ocular surface disease index (OSDI) scores and ocular itching questionnaire), slit-lamp examination of signs, tear break-up time (TBUT), corneal fluorescein staining (CFS), impression cytology (IC), tear total IgE levels, tear LT-α concentrations test and the expression of biomarkers in tears. Any adverse events, changes in visual acuity, and observation of ocular tolerance after treatments were recorded by researchers at each visit.

(1) The OSDI score ranged from 0 to 100 and was based on participants' self-evaluation. Itching scores ranged from 0 (none) to 10 (extremely severe). The specific ocular itching questionnaire is shown in Supplementary Material.

(2) Objective signs observed by slit-lamp (including conjunctival hyperemia, conjunctival edema, and conjunctival papillae) scales were 0-3 based on severity. TBUT was measured three times and the average value was recorded to demonstrate tear film stability. CFS score was obtained by dividing the cornea into four quadrants based on the *Chinese Dry Eye Expert Consensus (2020)*. 0 for no corneal

epithelial staining, 1 for 1-30 staining points, 2 for more than 30 without fusion, and 3 for punctate keratopathy fusion, corneal filiform paraphyte, and corneal ulceration.[18]

(3) Tear total IgE and LT-α levels test: Tear collection was performed 10 minutes following the TBUT examination. The 2.2 μL non-stimulated tears sample on the left eye was collected from the external ocular canthus using a disposable microcapillary fluid collector (2.2 μL; Seninda Biomedical Corporation, Guangzhou, China), then it was dispensed into the IgE test card and three total drops of buffer (Seninda Biomedical Corporation, Guangzhou, China) were added. The i-ImmunDx Analyzer was used to read the test card after it had rested undisturbed for 15 minutes. Meanwhile, determination of LT-α levels in tears was collected on the right eye and assigned to LT-α test cards.

(4) IC: Impression cytology analysis was performed on temporal bulbar conjunctiva by cellulose acetate filter paper (Sterlitech, Auburn, Washington, USA), subsequently fixed in paraformaldehyde, stained with periodic acid–Schiff and hematoxylin, and photographed with an x400 magnification. The goblet cell density was expressed as the number of goblet cells divided by the area (cells/mm^2). The degree was graded using Nelson grading criteria as well as the goblet cell density was determined by counting the cells per square millimeter.[19]

(5) Analysis of tear inflammatory cytokines: Collecting tears from both eyes by the method described above, avoiding reflex tearing if possible. Samples were then stored in a 0.5 ml centrifuge tube and frozen immediately at −80°C until analysis. Tears collected at each follow-up visit were further selected nine subjects (totally 72 tubes) in both groups separately. Subsequently, they were measured for ten cytokines concentrations by a microsphere-based immunoassay (Luminex, Austin, Texas, USA), including IL-5, IL-6, periostin, eotaxin-3, TNF-α, and MMP-9.

• Statistical analysis

SPSS 23.0 (IBM Corp, Armonk, NY, USA) software was used to analyze the data. The Kolmogorov-Smirnov test was used to verify the normality of the data. The Student's t-test or Paired samples t-test was used to evaluate normally distributed data, which were shown as mean ± standard deviation (SD); non-normally distributed data were analyzed with the Mann–Whitney U test or Wilcoxon signed rank test, and presented as median ± interquartile range (IQR). The associations between variables were calculated using Spearman's and Pearson correlation coefficient. GraphPad Prism 8.0 software was used to create the graphing. P<0.05 was considered statistically significant.

Results

The demographics and baseline features of the CsA and control group are shown in **Table 1.** There were no statistical differences in these parameters between the two groups at baseline. Three patients in the CsA group developed burning only within 1-3 days after applying the eye drops. No other adverse events were found.

• **Comparisons of the Subjective Symptoms in CsA and Control Groups**

At days 7, 30, and 60 after treatment, OSDI scores were significantly decreased in the CsA and control groups compared with baseline (all $P<0.001$). Additionally, OSDI scores were significantly lower in the CsA group than in the control group after day 60 of treatment ($P=0.039$).

Itching scores were considerably reduced in both the CsA and control groups at treatment days 7, 30, and 60 (all $P<0.001$) compared with the pre-treatment. Also, there were significantly lower in the CsA group than in the control group at all follow-up visits ($P=0.000$, $P=0.039$, and $P=0.031$) (**Table 2**).

• **Comparison of the Objective Signs Between Two Groups**

The changes in clinical parameters after treatment in CsA and Control groups are shown in **Figure 2**. Compared to baseline, conjunctival hyperemia scores were significantly reduced in both the CsA (all $P<0.001$) and control groups ($P=0.003$, $P<0.001$ and $P<0.001$) after days 7, 30, and 60 of treatment. However, there was no statistical difference in conjunctival hyperemia scores between the two groups at each time point

Conjunctival edema scores were significantly decreased in the CsA group at each follow-up visit from baseline (all $P<0.001$). However, a significant change in conjunctival edema scores in the control group only at day 60 of treatment ($P=0.003$). Apparent significantly lower conjunctival edema scores were observed in the CsA group than in the control group as early as day 7 after treatment initiation ($P=0.001$).

Conjunctival papillae scores were significantly reduced in the CsA group over time compared to baseline (all $P<0.001$). In the control group, however, it was significantly decreased at day 60 of treatment ($P<0.001$). Moreover, conjunctival papillae scores were significantly lesser in the CsA group than in the control group after treatment of day 30 and continued up to day 60 ($P<0.001$ and $P<0.001$).

TBUT was significantly increased in the CsA group after days 7, 30, and 60 of treatment from baseline ($P<0.001$, $P=0.023$ and $P<0.001$). Whereas in the control group, TBUT was observed to statistically significant increase after days 30 and 60 of treatment ($P=0.034$ and $P=0.005$). Simultaneously, there were significant differences in TBUT between the CsA and Control groups at each follow-up visit ($P=0.009$, $P=0.003$ and $P=0.005$).

CFS scores were markedly decreased in the CsA group at all follow-up visits compared to pre-treatment ($P=0.012$, $P=0.003$ and $P=0.007$). Obvious significant reductions in CFS scores were seen in the Control group after days 7 and 60 of therapy ($P=0.041$ and $P=0.030$). After day 30 of treatment, CFS scores were significantly lower in the CsA group than in the control group ($P=0.030$).

Tear total IgE concentrations were markedly declined in the CsA group after day 60 of treatment from pre-treatment ($P=0.017$), while there was no significant change in the control group across follow-up periods ($P=0.264$, $P=0.914$ and $P=0.648$). At each follow-up interval, there was no statistically significant difference in tear total IgE levels between the two groups ($P=0.593$, $P=0.051$ and $P=0.599$).

Compared with the baseline, the concentrations of LT-α in tears showed no statistically significant difference in the CsA group after days 7, 30 and 60 of treatment (P=0.107, P=0.133 and P=0.561). However, tear LT-α expression levels were markedly increased over time in the control group (P=0.001, P=0.002 and P<0.001). After day 60 of treatment, tear LT-α concentrations were markedly lower in the CsA group than in the control group (P=0.002).

After day 60 of treatment, goblet cell density was significantly increased in both the CsA and control groups from baseline (all P<0.001). However, there was no statistical difference in goblet cell density between the two groups after treatment (P=0.354).

• Changes in Tear Cytokines Expression Before and After Treatment in Two Groups

Tear cytokines expression levels were continuously monitored in 18 patients during each follow-up period (9 persons per group). After day 60 of treatment, IL-5 (P=0.008), IL-6 (P=0.036), periostin (P=0.010), eotaxin-3 (P=0.018) and MMP-9 (P=0.015) concentrations were shown the significant decrease over time in the CsA group compared to pre-treatment. In the control group, however, IL-5 (P=0.008), eotaxin-3 (P=0.007), TNF-α (P=0.008), and MMP-9 (P=0.015) were significantly reduced compared with those pre-treatments (**Figure 3**).

The changed values of tear cytokine levels before and after day 60 of treatment in the two groups are shown in **Figure 4**. All values decreased from the pre-treatment baseline. The changed values of IL-5 and periostin were significantly larger downregulation in the CsA group than in the control group (P<0.001 and P=0.026). However, the control group presented more decreased TNF-α levels (P=0.004). Although there was no statistical difference in the changed values of IL-6, eotaxin-3, and MMP-9 levels between the two groups, the changes of these cytokines in the CsA group showed a more declining trend than the control group (P=0.270, P=0.228 and P=0.465).

• Correlations Between Changes in Tear Cytokines and Changes in Clinical Parameters

Correlations between changes in tear cytokines levels and clinical parameters were analyzed, including tear total IgE, LT-α, IL-5, IL-6, periostin, eotaxin-3, TNF-α, and MMP-9. As shown in **Figure 5**, the changed values of tear total IgE were positively correlated with the change of OSDI scores after day 60 of treatment (R=0.321, P=0.019). And the changed values of tear total IgE levels showed a significant positive correlation with the change of itching scores at the 7th and 30th day of treatment (R_{7th}=0.368, P_{7th}=0.007; R_{30th}=0.295, P_{30th}=0.032).

Discussion

ACDE is a complicated condition that has been more common in recent years and has a severe impact on patients' quality of life. The pathogenesis is complex, and the disorders of AC and DE often coexist and reinforce each other.[6] The former can trigger DE by mechanisms such as damaging the epithelial barrier

of the ocular surface, causing tear film instability and meibomian gland dysfunction. In turn, DE can promote or aggravate AC mainly due to abnormal tear dynamics.[2,6]

Previous studies have demonstrated the therapeutic mechanism of CsA in DE is that it blocks the Ca^{2+}/calcineurin/nuclear factor of activated T cells (NFAT) signaling pathway to further limit T cell activation, and upregulates the levels of Fas/FasL and caspase to promote its apoptosis.[12,20] Also, CsA prevents the mitochondrial permeability transition (MPT) pore from opening by binding to cyclophilin D, which in turn increases the number of goblet cells and mucin secretion.[14,21] Other than that, CsA can reduce the allergic response by preventing mast cells (MCs) and basophils degranulation, and simultaneously suppressing eosinophil recruitment.[7] A recent study in mice suggested that calcineurin subunit A (CnAα) is involved in the early phases of mast cell-driven allergy reactions, and its deficiency resulted in the decrease of degranulation and inflammatory cytokines secretion in allergic responses.[22] According to these studies, we hypothesize that CsA may be effective against ACDE.

In this study, 0.05% CsA eye drops (II) monotherapy rapidly improved the symptoms (OSDI and itching scores) and objective signs (conjunctival hyperemia, conjunctival edema, conjunctival papillae, TBUT, CFS, and goblet cell density) in ACDE patients. In addition, the CsA group was more effective than the control group in improving ocular itching and TBUT at each follow-up period. And it also showed more reduction in conjunctival edema, CFS, and OSDI scores than the control group after days 7, 30, and 60 of therapy, respectively. This may be related to the respective different mechanisms of the medications' action. Olopatadine acts as a dual-action anti-allergic agent that both inhibits MCs degranulation and selectively antagonizes histamine H1-receptor.[23] Its combination with artificial tears is effective in relieving ocular itching and conjunctival hyperemia, replenishing tear volume. Whereas as mentioned above, CsA not only inhibits activated T cells and reduces inflammatory factors production such as IL-6 and MMP-9, but also inhibits T helper 2 (Th2) cells secretion of IL-4 and IL-5, decreases MCs degranulation and eosinophil recruitment.[7,24] In our study, tear IL-5, IL-6, periostin, eotaxin-3, and MMP-9 levels simultaneously decreased in the CsA group. Besides that, olopatadine inhibits tear secretion and reduces tear volume.[25] This side effect may make it less effective than CsA in improving TBUT. Furthermore, the preservative-free artificial tears are mainly used to replenish tear volume, while CsA can also promote mucin and tear secretion, prolong TBUT, and maintain tear film stability.[20] Therefore, CsA provides more significant efficacy in treating patients with ACDE, alleviating ocular allergy and improving ocular surface and tear function.

CsA is known to have different onset times in treating various ocular diseases. Studies have reported that CsA reduces signs and symptoms in VKC at 2–4 weeks and in moderate-to-severe DE or SS approximately at 1–3 months.[12,26,27] However, unlike previous perceptions of CsA, we noticed that 0.05% CsA acted rapidly in the early stage of treatment (around day 7) to relieve ocular itching. To find an answer, we further measured the levels of relevant tear cytokines. In this study, we observed a significant positive correlation between the changed values of tear total IgE levels and the changes in itching scores at the 7th and 30th day of treatment. Also, tear total IgE levels showed a significant decrease in the CsA

group. The control group, however, experienced no significant change in tear total IgE. Cui and colleagues [28] revealed the novel mechanism that allergic ocular itching can be caused by the direct activation of sensory neurons Fc-epsilon receptor I (FcεRI) by the IgE-immune complex, which is independent of the classical mast cell pathway. IgE upregulates sensory neurons FcεRIα, which acts directly on conjunctival itchy sensors to exacerbate itching. This way of neuromodulation is usually faster than the signaling process between inflammatory factors. Therefore, the rapid alleviation of ocular itching by CsA may be mediated by the decrease of tear IgE levels to affect this neuroimmune axis. Additionally, Ambroziak [29] mentioned that CsA exhibits a mildly inhibitive effect on B-cell by limiting the induction phase of lymphoid cell proliferation, and may thereby decline to tear total IgE levels. Our results further support this finding.

Moreover, periostin levels markedly decreased in the CsA group and showed more decline than those in the control group. Periostin, a downstream molecule of IL-4 and IL-13, can directly contribute to the stimulation of sensory nerve fibers to induce ocular itching, along with indirectly causing itching by binding to α_V integrins to promote the secretion of histamine by MCs, basophils, and eosinophils.[30,31] The significant reduction of periostin in the CsA group may be another reason for such an alleviation in ocular itching. However, most of the periostin values measured at days 7 and 30 after treatment in both groups were less than the kit's recommended limit range, and no exact valid values were presented in this study. Although the results of this assay also support to some extent our inference above, these two time periods were not analyzed based on the principle of rigor in analyzing data. And we will further test this period in the following study.

In this study, we also measured allergy-associated cytokines such as IL-5, periostin, and eotaxin-3 produced by the Th2 cells pathway, as well as pro-inflammatory factors in DE, such as TNF-α and IL-6 expressed mainly by Th1/Th17 cells.[32,33] This study showed that IL-5 and eotaxin-3 levels were significantly reduced after therapy in both groups, and compared to the control group, the changes of IL-5 were more downregulated in the CsA group. Interestingly, we found that IL-6 levels in the CsA group and TNF-α levels in the control group markedly declined over time compared with baseline, respectively. Previous studies also suggested that CsA significantly reduced IL-6 concentrations but not TNF mRNA.[34] This is in line with our results. Moreover, Cook and associates[35] reported that olopatadine blocks the release of TNF-α from human conjunctival MCs and decreases TNF-α levels in a concentration-dependent way. In addition, it has been suggested that MCs also release TNF-α, MMP-9, and other chemokines in addition to histamine. MCs are the main potential source of TNF-α.[36] In the present study, the changed values of the reduction in TNF-α levels in the CsA group were significantly less than that in the control group, which may also indicate that CsA mainly acts on T cells with relatively weak inhibition of cytokines downstream of MCs.

Furthermore, MMP-9 has been receiving much attention as a crucial indicator for diagnosing and monitoring DE, while the role of LT-α has gradually attracted more attention in recent years.[37] LT-α, namely TNF-β, is involved in the pathogenesis of ocular diseases such as DE, SS, and oGVHD.[38–40] Decreased

tear LT-α levels imply impaired immune regulation and increase DE susceptibility. In this study, MMP-9 concentrations markedly decreased after treatment in both groups, but the changed values were no statistical distinction between the two groups. MMP-9 accelerates the degradation of the corneal extracellular matrix and apoptosis of epithelial cells.[41] Evidence from abundant studies showed that MMP-9 levels were elevated in both dry eye and VKC patients and reduced after topical CsA treatment.[41,42] These findings are consistent with our results. Additionally, we found that tear LT-α levels significantly increased in the control group with increasing treatment duration. However, LT-α levels was no statistical difference in the CsA group although there was a tendency to rise. And tear LT-α levels in the CsA group were significantly lower than that in the control group at day 60 after treatment. Kuprash [43] explored the impact of CsA on LT-α and LT-β expression in human peripheral blood mononuclear cells and found that CsA significantly inhibited the expression of LT-α mRNA and protein by affecting NFAT signaling. Therefore, this may explain why the expression levels of LT-α in the CsA group were lower than that in the control group.

Limitations are inevitable in this study. Firstly, the relatively small amount of tear volume limited us from doing more tear cytokines analyses, future research should expand participants and involve as many tear sample tests as possible. Secondly, no comparative assessment of the efficacy of different dosages of CsA was performed in this study to determine its optimal administration, we will address this shortcoming in future studies.

In conclusion, this study confirms that 0.05% CsA eye drops (II) monotherapy four times daily can rapidly improve the signs and symptoms, especially in ocular itching and TBUT, in patients with ACDE. And its efficacy is superior to 0.1% olopatadine combined with artificial tears. Additionally, CsA can downregulate the expression levels of tear inflammatory cytokines, such as tear total IgE, IL-5, IL-6, periostin, eotaxin-3, and MMP-9. Among them, the rapid relief of ocular itching by CsA may be associated with the downregulation of tear total IgE and periostin levels. These findings raise a new therapeutic option in ACDE. Further long-term studies with larger samples are needed to provide more evidence and reference value to clinicians in treating patients with ACDE.

Declarations

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COMPETING INTERESTS

The authors declare no competing interests.

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Tables

Table 1. Demographic characteristics and evaluation indicators of the two groups.

	CsA (n=28)	Control (n=25)	*P*-value
Age, years	34.43±8.41	38.28±12.62	0.193
Gender, n (%)			0.562
Male	11 (39.29%)	7 (28.00%)	
Female	17 (60.71%)	18 (72.00%)	
OSDI	21.13±10.95	23.57±12.76	0.458
Itching scores	8.00±1.00	8.00±3.00	0.200
Conjunctival hyperemia	2.00±0.75	2.00±1.00	0.197
Conjunctival edema	1.00±1.75	0.00±1.00	0.078
Palpebral conjunctiva papillae	2.00±1.00	2.00±0.50	0.052
TBUT, sec	3.89±1.73	4.00±2.00	0.920
Corneal fluorescein staining	0.00±1.00	0.00±1.50	0.902
Tear total IgE (IU/mL)	6.45±5.08	1.53±6.15	0.059
Tear LT-α levels (ng/mL)	0.36±0.88	0.43±0.59	0.662
Goblet cell density (cells/mm^2)	134.42±136.45	81.02±168.90	0.630

Normally distributed data are expressed as mean ± standard deviation (SD), and non-normally distributed data are presented as median ± interquartile range (IQR). *P* values for comparisons of clinical parameters between the two groups were determined using the Mann-Whitney U test and Student's t-test, respectively.

Table 2. Comparisons of subjective symptoms before and after treatment between two groups.

	CsA (n=28)	Control (n=25)	*P*-value
OSDI			
Baseline	21.13±10.95	23.57±12.76	0.458
7 days	10.80±15.60	14.13±8.57	0.742
30 days	9.05±14.38	11.27±7.88	0.887
60 days	6.90±7.48	10.66±5.69	**0.039**
P-value			
Baseline vs. 7 days	**< 0.001**	**< 0.001**	
Baseline vs. 30 days	**< 0.001**	**< 0.001**	
Baseline vs. 60 days	**< 0.001**	**< 0.001**	
Itching scores			
Baseline	8.00±1.00	8.00±3.00	0.200
7 days	2.00±2.75	4.56±1.80	**< 0.001**
30 days	1.00±2.75	2.00±4.50	**0.039**
60 days	0.00±0.22	0.70±1.00	**0.031**
P-value			
Baseline vs. 7 days	**< 0.001**	**< 0.001**	
Baseline vs. 30 days	**< 0.001**	**< 0.001**	
Baseline vs. 60 days	**< 0.001**	**< 0.001**	

Data were presented as mean ± standard deviation (SD) or median ± interquartile range (IQR). Paired t-test or Wilcoxon signed rank test was used for comparison within the CsA and control groups. Student's t-test or Mann–Whitney U test was selected for comparisons between the two groups. Bolded font indicates statistical differences.

Figures

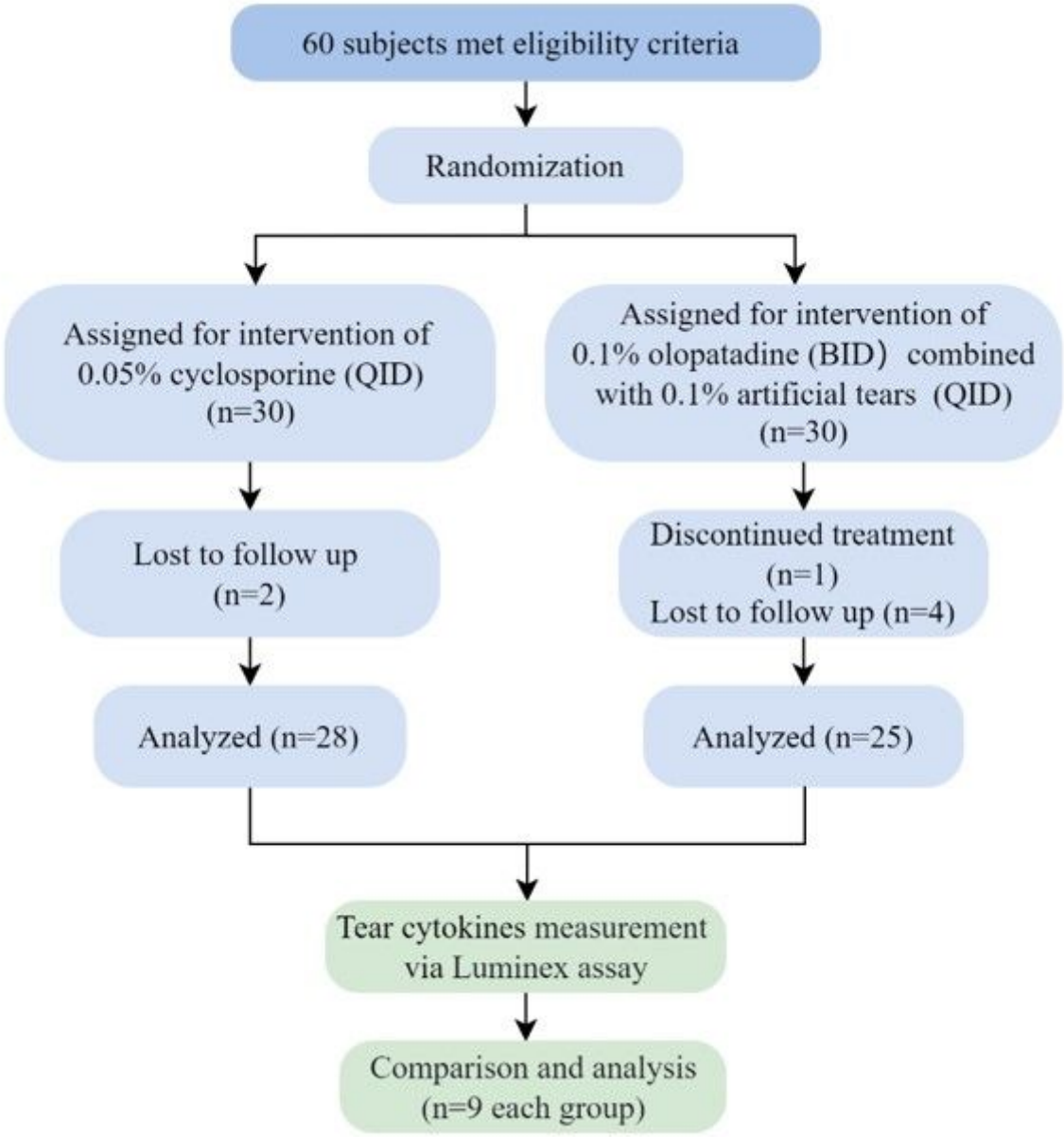

Figure 1

Flowchart of a randomized controlled trial assessing the clinical efficacy of 0.05% CsA eye drops (II) in allergic conjunctivitis-associated dry eye. BID=twice daily; QID=four times daily.

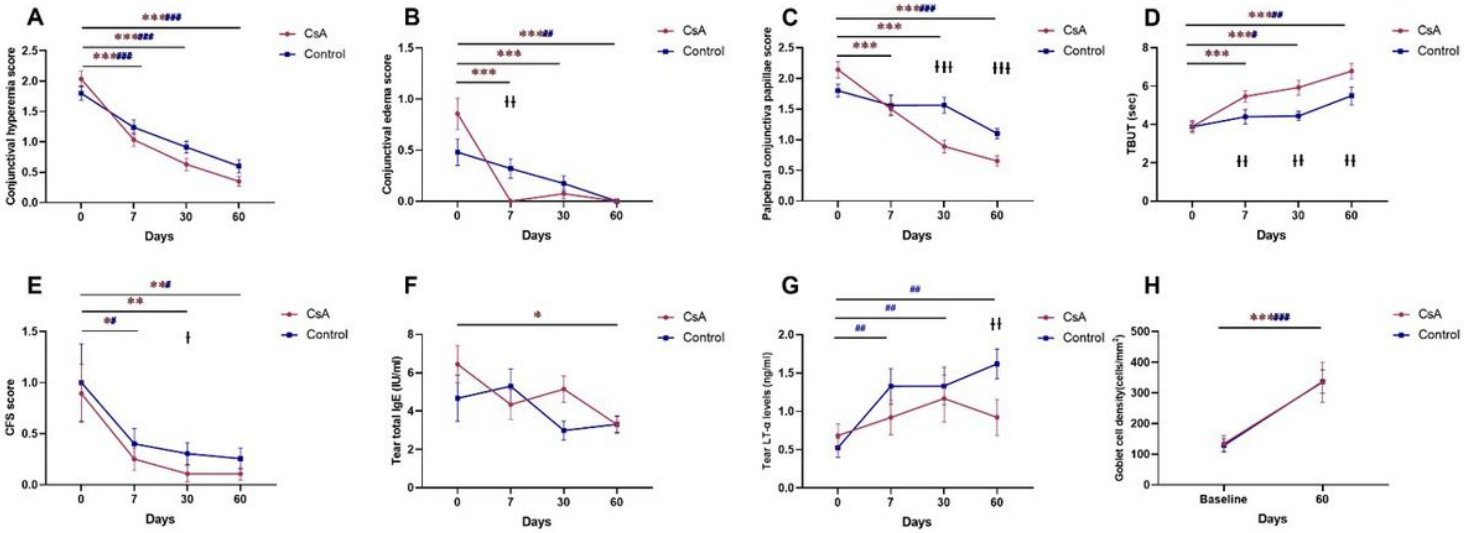

Figure 2. Comparison of changes in clinical parameters in patients with ACDE after treatment (**A-H**). Data are presented as the mean±standard error of the mean (SEM). The mean values of the CsA group and the control group are shown in the red rounds and blue squares, respectively. *Indicates significant differences within the CsA group compared with baseline, **#**represents markedly differences within the control group from baseline, † indicates statistically significant differences between the two groups. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ (**#** and † symbols as above).

Figure 2

See image above for figure legend

Figure 3. A. Tear cytokines Profiles in the CsA group before and after 60 days of treatment. **B.** Tear cytokines expression levels in the control group at pre-treatment and post-treatment. Paired t-test and Wilcoxon signed rank test were selected for comparison within the CsA and control groups. * represents statistical differences between pre-treatment and post-treatment day 60. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

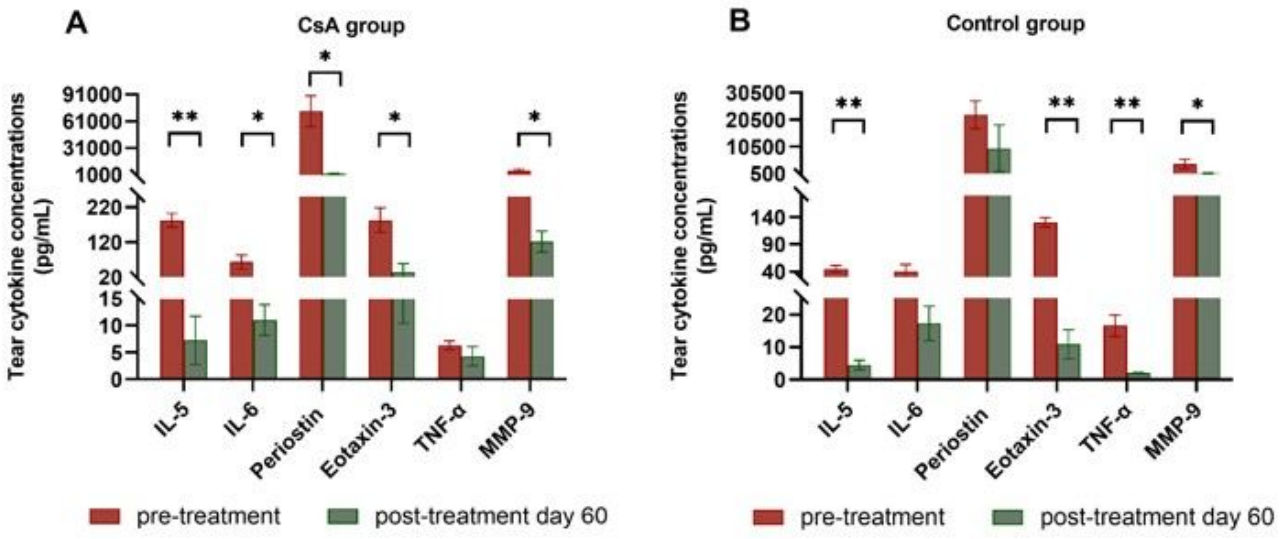

Figure 3

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Figure 4. Changes in Tear Cytokines Concentrations between the CsA group and the control group (**A-F**). The changed values of IL-5 (**A**) and periostin (**C**) were significantly larger downregulation in the CsA group than in the control group. The changes in TNF-α levels were more decreased in the control group than in the CsA group (**E**). Student's t-test and Mann–Whitney U test were selected for comparisons between the two groups. *indicates significant differences between the two groups. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

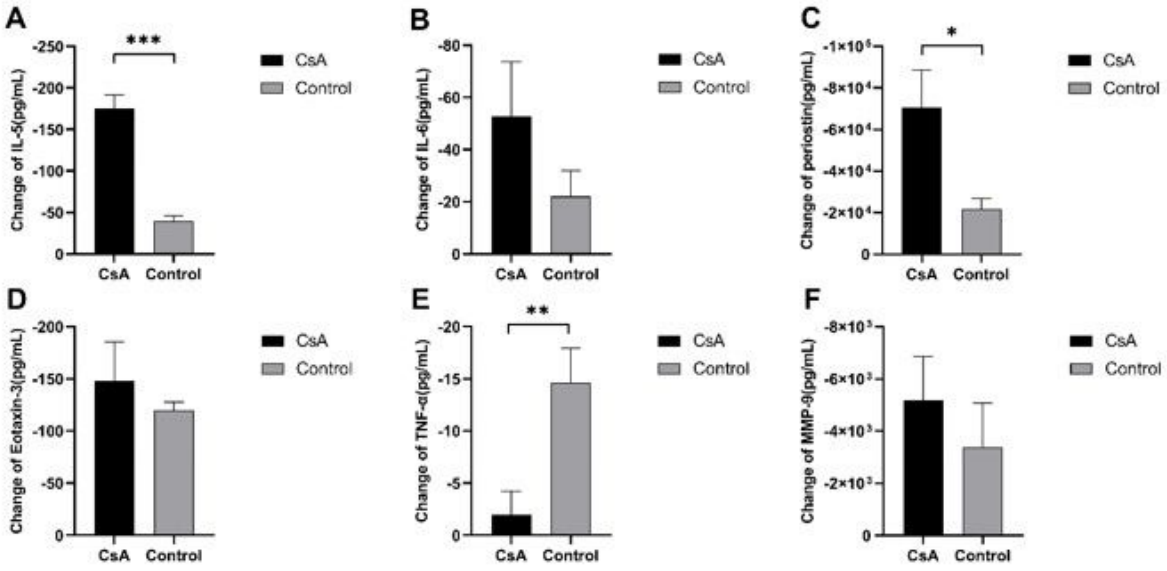

Figure 4

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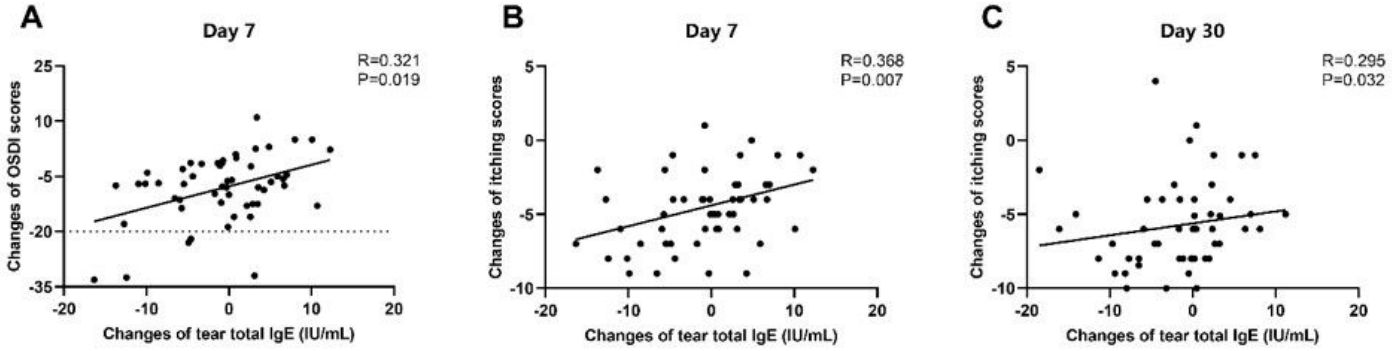

Figure 5

Scatter plot of the correlation between the changed values in tear total IgE and the changes in OSDI scores (**A**) as well as itching scores (**B-C**) at different treatment periods. The changed values were subtracted the baseline value from the post-treatment value and analyzed by using Spearman's correlation analysis.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryMaterial.docx