Original Article

Safety and Efficacy of Cyclosporine (0.05% versus 0.09%) in Dry Eye Disease. Is it the Strength of Cyclosporin that Really Matters?

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ABSTRACT

Introduction: This is a multicenter, randomized, interventional, double masked study aimed to compare safety and efficacy of cyclosporine (0.05% versus 0.09%) in dry eye disease.

Materials and methods: Random allocation of patients (n=450) was done in two groups by parallel assignment (1:1). Group1(n=225) received CAs 0.05% drops twice daily, and group 2 (n=225) received CAs 0.09% drops twice daily for 3 months. Primary outcomes were changes from baseline in Lissamine green staining score, Nelson grade on conjunctival impression cytology and tear film osmolarity. Secondary outcomes were changes in dry eye symptom score. Schirmer's test scores, changes in corneal fluorescein staining and changes in tear film break up time.

Results: Within the groups, there was a significant improvement (ANOVA, P<0.05) in tear film osmolarity, lissamine green staining score, dry eye symptom score, corneal fluorescein staining and Schirmer test scores over 3 months of intervention. However, the difference in Nelson Grade, goblet cell density, and tear film break-up time was not statistically significant. Between the groups, there was a significantly better improvement in tear film osmolarity (ANOVA, P<0.001), Lissamine green staining score (ANOVA, P=0.002), corneal fluorescein staining (ANOVA, P=0.011), dry eye symptoms (ANOVA, P=0.040) and Schirmer test scores (ANOVA, P=0.001) with CAs 0.09%. However, the improvement in Nelson grade, tear film break-up time was not significantly different between the two groups. The overall patient's comfort was significantly better over time in patients on CAs 0.05% (ANOVA, P<0.001).

Conclusion: Increasing strength of CAs better improves corneal staining, tear production, tear film osmolarity but not conjunctival morphology and tear film stability.

Key words: Aqueous solution, CAs, Dry eye disease, Emulsion, Lissamine Green staining.

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INTRODUCTION

Dry eye or keratoconjunctivitis sicca is a complex inflammatory disorder involving the ocular surface; tears lose their ability to maintain homeostasis, leading to tear film instability, increased tear film osmolarity, and ocular surface damage due to inflammation (Craig et al., 2017).

Prevalence of dry eye disease worldwide has wide variability; the reported prevalence ranges from 5 to 50% (Paulsen et al., 2014). Patients which report may represent the 'tip of the iceberg' as patients with milder forms of the disease often do not seek expert opinion. Thus, it may not be possible to estimate the true prevalence of dry eye in this subset of patients. In the Indian subcontinent, prevalence of dry eye disease in the national capital region was found to be about 32% (Titiyal et al., 2018).

The management of dry eye is challenging not only because of its multifactorial etiology but also due to symptom sign discordance (Kumar et al., 2014). Despite these limitations, the goal of dry eye treatment is to restore ocular surface homeostasis by halting the vicious circle of ocular surface inflammation and tear film instability.

Ocular surface inflammation is critical to the pathophysiology of dry eye; therefore, it is logical to consider anti-inflammatory agents for dry eye treatment (Bron et al., 2017). CAs is an anti-inflammatory and an immunomodulatory drug that was originally used to prevent rejection after organ transplantation. It affects immune function by interfering with the activity and growth of T cells; it inhibits calcineurin, thereby preventing T lymphocytes activation and subsequent release of inflammatory mediators like IL-2 and other cytokines (Fruman et al., 1992).

Several studies have demonstrated the efficacy of CAs 0.05% emulsion in reducing corneal staining and increasing tear production in patients with dry eye disease. However, only a few clinical trials have explored the efficacy of increasing strength of CAs (0.09%) on these parameters. Some studies have also evaluated the efficacy of CAs 0.05% in increasing conjunctival goblet cell density, but results have been inconsistent (Baudouin et al., 2017; Kunert et al., 2002). As there is a high prevalence of dry eye in the urban north Indian population (32%), the present study was designed to compare the safety, efficacy, and patient satisfaction between CAs 0.05% ophthalmic emulsion and CAs 0.09% nanomicellar aqueous solution for treatment of keratoconjunctivitis sicca.

MATERIALS AND METHODS

A multicenter, randomized, double masked study was done at three referral teaching hospitals in the northern part of the subcontinent. Approval of the institutional ethics committee was obtained, and the trial was registered with a clinical trial registry [UMIN:000035991]. The tenets of declaration of Helsinki were followed while obtaining written informed consent from all participants.

Eligibility criteria

Subjects greater than 18 years of age who were diagnosed with dry eye based on corneal staining (score >3 or <9), and/or dry eye symptoms (score >1) were included in the study. Dry eye symptoms were evaluated based on response of subjects to the Indian dry eye questionnaire (Dry Eye Scoring System, DESS ©) (Table 1). DESS has been recently validated in a multicenter study done at our center; a high level of internal consistency was observed as compared to OSDI, as determined by a Cronbach's alpha of 0.863.

DESS is an eighteen point questionnaire which characterizes dry eye patients based on severity (symptom free, mild, moderate, and severe). A score of 0 to 3 was assigned to dry eye symptoms such as blurring of vision, itching, or burning, sandy or gritty sensation, and redness, respectively. In absence of symptoms, the score was (0), when sometimes present (1), frequently present (2), and present most of the time (3). Collectively, symptoms score of 0–6 was considered mild, 6.1– 12 moderates, and 12.1–18 severely symptomatic dry eye, respectively. The minimum score for inclusion in the study was (i.e., any symptomatic patient) one. (Bhargava et al.,2015; Bhargava et al., 2015; Bhargava et al., 2016; Bhargava et al., 2016).

Exclusion criteria

Patients on topical CAs 0.05% or oral omega 3 fatty acids within the last three months of the baseline visit were excluded. Patients using punctal plugs, history of contact lens wear within 3 months of study enrolment were excluded. Use of topical or systemic medications for (glaucoma, allergy, infection, etc.) within 4 weeks that could interfere with the tear film tests/study results were excluded. Any history of ocular surgery within the last 6 months, eye infection/allergy, pterygium, eyelid pathology (e.g., trichiasis and entropion), and CAs hypersensitivity were also excluded.

Table 1: Indian dry eye questionnaire and scoring (DESS©).

Symptom	Score (maximum 18)			
	Absent (0)	Sometimes present (1)	Frequently present (2)	Always present (3)
Itching or burning				
Sandy sensation				
Redness				
Visual blurring				
Eye fatigue				
Excessive blinking				

[a]Scores of 0 to 6 were mild, 6.1 to 12 were moderate, and 12.1 to 18 indicated severely symptomatic dry eye. 23-26 ©Bhargava R, India.

Randomization, masking and sample size calculation

Sample size was calculated using a web-based calculator of the University of British Columbia which can be accessed using the link [https://www.stat.ubc.ca/~rollin/stats/ssize/n1.html]. To calculate the sample size, we compared the mean difference in tear film osmolarity (primary outcome measure) between the two groups by conducting a pilot study on 10 subjects. The mean reduction in tear film osmolarity in CAs 0.05% group was 15.4 and in CAs 0.09% group was 20.6, respectively. The common SD was 0.45. Assuming 1:1 randomization, 90% power (alpha = 0.05), and a precision error of 5% to detect difference of 20% or more in tear film osmolarity between (CAs 0.05% and CAs 0.09%), the estimated sample size in each group was 230.

Trial groups

Consecutive patients with dry eye were randomly allocated to one of the two groups by a parallel assignment (1:1). The allocation codes were generated by a web-based programme and was stratified according to the research center with a permuted block method with randomly chosen block sizes. The generated codes were sealed in red envelopes and were opened by investigators who were not involved in patient care. Patients in Group 1 received CAs 0.05% drops twice daily and patients in Group 2 received CAs 0.09% drops twice daily for 3 months. The subjects were blinded to the contents of eye drops. The two types of eye-drop bottles resembled each other. On monthly visits, subjects returned the empty bottles and replacement eye drops were provided to them. The frequency of the regimen was reduced or suspended in cases when patients reported any symptoms or when there was a contraindication to treatment to any of active eye drops. The patient could restart or resume the regimen with resolution of symptoms or contraindications. In the CAs 0.09% group, ten patients could resume treatment after resolution of symptoms with once daily dosing. Thereafter, normal dosing schedule was restored as tolerance developed, and treatment completed successfully without further discontinuation.

Tear film tests

As all the tests were planned between 10 AM and 12 PM, the participants were instructed to visit the dry eye clinic in the morning. Patients were advised not to use artificial tear preparations, 2 hours prior to testing. At each examination, subjects underwent TBUT, tear film osmolarity, Lissamine green staining, corneal fluorescein staining, conjunctival impression cytology (CIC), and Schirmer test, respectively. Furthermore, the subjects were administered the dry eye questionnaire at each monthly visit. The information obtained from the questionnaire was concealed from the independent investigator.

Tear film break up time was first measured as excessive manipulation of the eyelids at this stage could lead to erroneous results. A sterile

fluorescein strip containing 1 mg fluorescein sodium (Madhu Instruments, Delhi, India) was applied over the inferior bulbar conjunctiva after moistening with normal saline solution. Normal blinking was encouraged (without squeezing) to evenly distribute the fluorescein. The tear film was observed under a slit lamp using a cobalt blue filter. The interval between the last complete blink and the first appearance of a dry spot on the cornea was measured with a timer. A total of three readings were taken in succession and averaged (Lemp MA., 2015).

The subject then waited for another 30 minutes, and a Schirmer test with anaesthesia (0.4% oxybuprocaine hydrochloride) was performed with eyes closed.

Subjects waited for another 30 minutes; Lissamine green staining was done with 10μL of 1% solution. Staining was scored using white light and then a Hoya 25A red barrier filter during slit lamp biomicroscopic illumination with white light. Staining scores were recorded according to the Oxford scheme (Wu et al., 2019).

CIC was performed after anesthetizing the eye with 4% xylocaine. A cotton-tip applicator was used to dry the lacrimal lake at the inner canthus. A circular 0.2-mm filter paper measuring 13 mm in diameter (Sartorius, Gottingen, Germany) was applied over the inferior bulbar conjunctiva using blunt-tipped forceps. The non-exposed conjunctiva was used to obtain CIC samples to eliminate the environmental influences on the ocular surface in the exposed part. Gentle pressure was applied on a paper strip with a glass rod held in the other hand. It was then removed in a peeling fashion 4 to 10 seconds later; the specimen thereafter transferred to the laboratory for fixation (ethyl alcohol, formaldehyde, and glacial acetic acid in 20:1:1 volume ratio) and staining. Periodic acid–Schiff staining and counterstaining with hematoxylin and eosin was done after keeping the slide at room temperature. The stained slide was then examined under a light microscope with X100 low power field (X10 objective lens). After localization of cells, examination was done under X400 final magnification (X40 objective). Goblet cells and epithelial cells were counted in at least 10 HPF (Nelson JD., 1983).

Outcome measures / study endpoints:

Primary outcome measures

Changes from baseline in tear film osmolarity, Lissamine green conjunctival staining score (an assessment of corneal epithelial cell disruption, higher scores indicative of more disruption), and Nelson grade (a measure of nuclear cytoplasmic ratio of epithelial cells and goblet cell density on conjunctival impression cytology) at 3 months were primary outcome measures.

Secondary outcomes measures

Secondary outcome measures were changes in Schirmer's test scores (a measure of tear volume), changes in corneal fluorescein staining and changes in tear film break up time (a measure of tear film stability), and dry eye symptoms at 3 months.

Patient comfort and safety

Slit lamp examinations were conducted, Snellen VA was measured, and adverse events were recorded at all follow up visits. The comfort/ tolerability of the study medications was evaluated at day one, day seven, day thirty and at 3 months. This was graded on a scale of 0-4; a score of 0= implied no discomfort, 1=mild, 2=moderate, 3= severe and 4=very severe discomfort, respectively).

All participants were prescribed carboxymethylcellulose 0.5% eye drops four times daily for 10 days, during the run-in period.

Statistical methods

IBM, SPSS Statistics version 27 (IBM Inc.) was used for statistical analysis. One eye of each patient was randomly selected for examination and subsequent evaluation. Group similarities at baseline were ensured with Independent t tests. Differences in mean test values over the course of 3 months of treatment were evaluated with one-way repeated-measures analysis of variance (ANOVA). The values used for assessing change were the means of values obtained during the 2-month and 3-month visits; if a value from only one of these visits was available, that value was used. Comparisons of the mean change in continuous measures between trial groups and associated 95% confidence intervals were based on linear regression with a robust variance estimator. Log-rank test was used to evaluate differences between trial groups in the cumulative proportion of patients with an adverse event.

RESULTS

A total of 607 patients were screened for eligibility for study participation. Four hundred and sixty-seven (467) patients were found eligible as 140 patients could not meet the inclusion criteria. Three patients in group 1 and 7 patients in group 2 (10 patients) declined to participate in the study. Four patients in group 1 and 3 patients in group 2 (seven patients) were lost to follow up. Therefore, 450 patients were analyzed for results statistically, out of which 225 patients were assigned to the CAs 0.05% (Group 1) and 225 patients to the CAs 0.09% (Group 2), respectively. Although, three patients in group 1 and ten patients in group 2 had adverse effects severe enough to warrant discontinuation of treatment but were included for assessment as they completed two months of therapy. Patients' enrolment, withdrawal, adverse events, and follow-up is mentioned in Figure 1.

The mean age of patients in Group 1 was 56.4±5.71years and in Group 2 was 56.1±5.6 years, respectively (paired t-test, P= 0.123). The gender was comparable between the two groups (Chi-square tests, P=0.167). In group 1, 108(48%) had moderate and 93 (42%) had severe dry eye and in group 2, 100(44%) had moderate and 105(47%) had severely symptomatic dry eye, respectively (Chi-square test, P= 0.196). In baseline characteristics, there were no significant imbalances between trial groups (Table 2).

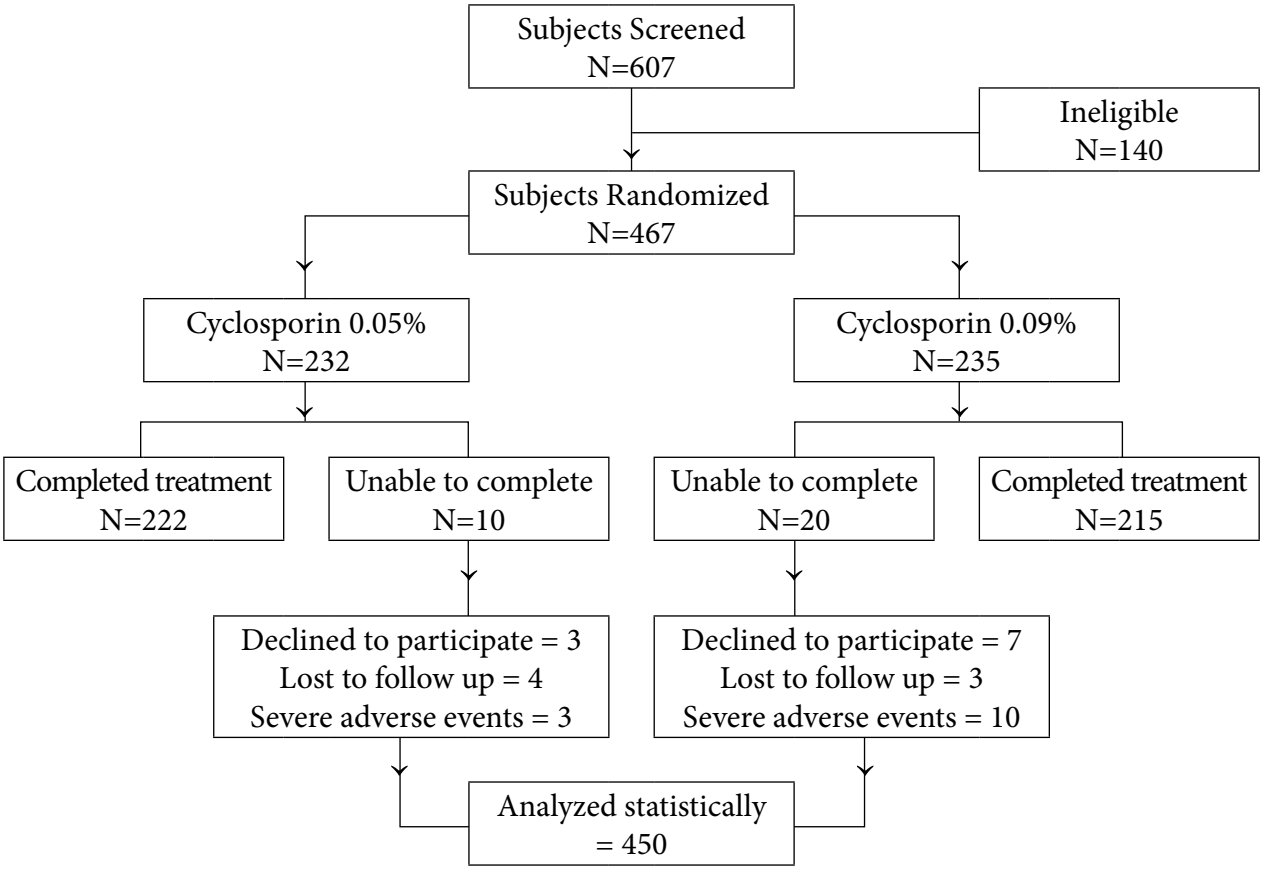

Figure 1: Flow chart showing patients enrolment, withdrawal, adverse events, and follow-up.

Table 2: Baseline characteristics.

VARIABLE	GROUP 1	GROUP 2	P-value
Age (Years)	56.4±5.71	56.1±5.6	0.123
Lissamine Green Score	5.9±0.24	5.8±0.27	0.341
Tear film Osmolarity (mOsm /L)	323±3.4	326±4.5	0.169
Nelson Grade	2.05±0.18	2±0.16	0.096
GCD (cells/mm2)	440±30.3	445±30.6	0.842
DESS Score	9.4±1.6	9.2±1.8	0.645
Dry eye Severity			
Mild	24	20	0.196*
Moderate	108	100	
Severe	93	105	
Schirmer (mm)	8±0.5	8.4±0.6	0.121
TBUT (sec)	8±1.6	8.6±1.3	0.708
CFS Score	4.6±0.24	4.72±0.28	0.098

*GCD (Goblet Cell Density), DESS (Dry Eye Scoring System), CFS (Corneal Fluorescein staining), TBUT (Tear Film Break up Time), *Chi-square tests.*

Within group comparisons

Group 1 (CAs 0.05%): On repeated measure ANOVA, there was a significant improvement in tear film osmolarity, lissamine green staining score, dry eye symptom score, corneal fluorescein staining and Schirmer test scores over 3 months of intervention (ANOVA, P=0.010, 0.020, 0.045, 0.050 and 0.010, respectively). However, the difference in Nelson Grade, goblet cell density, and TBUT was not statistically significant over time (ANOVA, P=0.345, 0.768 and 0.645, respectively). At study endpoint, 15 (6.7%) patients had severe, 20 (8.9%) moderate and 5(2.2%) mild dry eye symptoms, respectively.

Group 2 (CAs 0.09%): On repeated measure ANOVA, there was a significant improvement in tear film osmolarity, lissamine green staining score, dry eye symptom score, corneal fluorescein staining and Schirmer test scores over 3 months of intervention (ANOVA, P=0.001, 0.006, 0.040, 0.014 and 0.001, respectively). However, the difference in Nelson Grade, goblet cell density, and TBUT was not statistically significant over time (ANOVA, P=0.246, 0.575, and 0.446, respectively). At study endpoint, 25(11.1%) had severe, 24 (10.7%) moderate and 9(4%) mild dry eye symptoms, respectively.

Between group comparisons

At study endpoint (3 months post intervention), there was a significantly better improvement in tear film osmolarity (ANOVA, P<0.001), Lissamine green staining score (ANOVA, P=0.002), corneal fluorescein staining (ANOVA, P=0.011), and Schirmer test scores (ANOVA, P=0.001) with CAs 0.09% (Figures 2a, b, c, d, and e), respectively.

On post hoc analysis, a significantly higher proportion of participants had improvement of tear film osmolarity, Lissamine green staining scores, CFS and Schirmer test scores with CAs 0.09% as compared to CAs 0.05% (21%vs16%, 24% vs 14%, 18% vs 14%, and 43% vs 34%,), respectively.

However, the improvement in Nelson grade (ANOVA, P=0.407), goblet cell density (ANOVA, P=0.183), tear film break-up time (ANOVA, P=0.880) and dry eye symptoms (ANOVA, P= 0.096) did not differ significantly between CAs 0.05% and CAs 0.09% at study end point (Figures 3a, b and c), respectively.

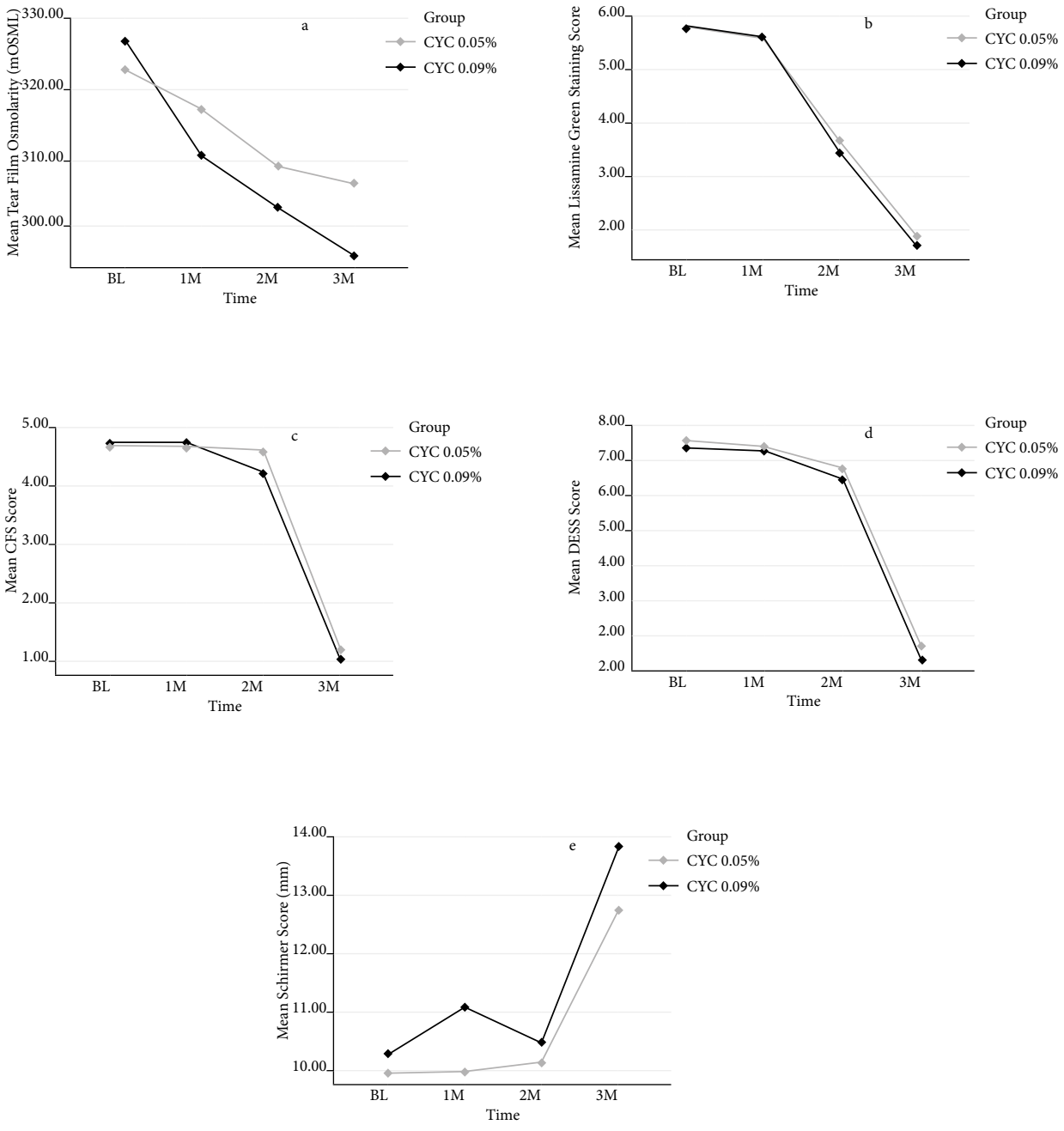

Figure 2: Line diagram showing mean change in Tear film osmolarity, Lissamine Green staining scores, corneal fluorescein staining, dry eye symptom score and Schirmer test scores between CAs 0.05% and CAs 0.09%, respectively.

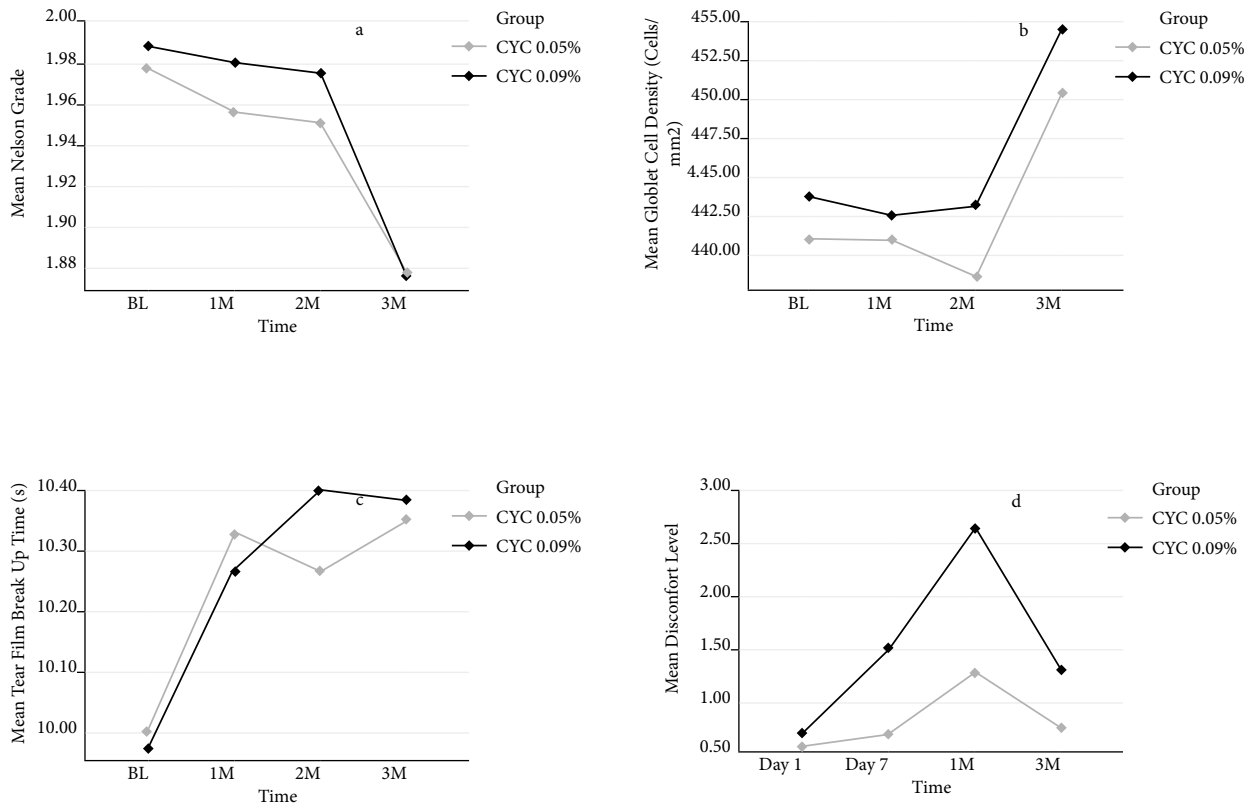

Figure 3: Line diagram showing mean change in Nelson grade, Goblet cell density, tear film break up time, and patient comfort levels between CAs 0.05% and CAs 0.09%, respectively.

Patient comfort level

Discomfort with medication had a biphasic response. There was an initial peak at day seven followed by a sharp rise over 1 month in both treatment groups. This was followed by a sharp fall over the next two months (Figure 2d). The overall patients comfort level was significantly better over time in patients on CAs 0.05% (ANOVA, P<0.001).

Adverse effects

Twenty-two (9.7%) patients in group 1 (CAs 0.05%) and 40 (17.8%) patients in group 2(CAs 0.09%) reported severe burning, pain and redness in eyes following topical instillation (discomfort level 3 to 4). The symptoms peaked at 1 month in both treatment arms. Twenty patients on CAs 0.09% had severe symptoms and were unable to continue medication beyond 2 months. However, ten patients out of these could resume treatment after resolution of symptoms with once daily dosing. Thereafter, normal dosing schedule was restored as tolerance developed, and treatment completed successfully without further discontinuation.

DISCUSSION

Initial CAs formulations that were oil-based had low ocular surface bioavailability. Second, these formulations were associated with several side effects like vision blurring, burning and stinging sensation, and lastly, they were poorly tolerated by patients. These limitations have led to development of advanced modalities with enhanced drug delivery to the ocular surface. Newer formulations containing higher concentrations of CAs (0.09%) claim to deliver therapeutic concentrations of CAs with minimal discomfort to patients. CAs 0.05% in an oil in water emulsion and CAs 0.09% in a preservative free nanomicellar aqueous solution have now been approved for dry eye management.

In this study we directly compared a higher concentration of CAs (0.09%) aqueous solution with CAs 0.05% ophthalmic emulsion to treat dry eye disease. Our results revealed that twice-daily dosing of CAs 0.09% led to statistically significantly better improvement in tear film osmolarity, corneal staining, dry eye symptoms and tear production than CAs 0.05% after 3 months of treatment. However, improvement in goblet cell density and tear production was not statistically significantly different between the two strengths of CAs. Second, adverse effects and patient discomfort were significantly higher with higher strength of CAs.

There exist discrepancies between reported studies in literature on whether CAs at a higher dose (>0.05%) is better than CAs 0.05%. A randomized, controlled study (Baiza-Duran et al., 2010) reported that aqueous CAs 0.1% was superior to 0.05% in alleviating ocular dryness, photophobia, and ocular fatigue but not for other symptoms, like tearing or foreign-body sensation. On the contrary, another randomized controlled study (Sall et al., 2000) found that 0.05% CAs emulsion was superior to CAs 0.1% in improving symptoms of blurred vision only.

The present study found that treatment with higher concentration (0.09%) CAs significantly improved corneal staining and tear production. The present work however, further explored changes in conjunctival epithelial cell morphology, goblet cell density and tear film stability; the two treatment arms did not differ in these parameters after 3 months of treatment. Moreover, 17.8% in group 1 and 25.7% patients in group 2 had dry eye symptoms at 3rd treatment month despite significant improvement in signs of dry eye disease (corneal staining, tear production and osmolarity). The inconsistencies in symptom improvement between studies may be partly explained by the symptom sign discordance that exists in dry eye disease and partially by the presumed irritative nature of CAs (Nichols et al., 2004; Kyei et al., 2018; Bartlett et al., 2015).

Undoubtedly, one of the major causes for noncompliance or discontinuation of treatment is poor drug tolerability. However, it still is unclear whether drug intolerability with CAs is due to its formulation (emulsion/aqueous solution) or the drug itself. Studies claim that

nanomicelles enhance drug delivery to the ocular surface and have better tolerability and bioavailability (Mandal et al., 2019; Mandal et al., 2017). Recently a study with three treatment arms compared the safety and efficacy of CAs 0.09%, 0.05% and vehicle, respectively, for dry eye management; the authors found nanomicelles CAs (0.09%) to be more effective and better tolerated at a higher strength as compared to CAs 0.05% or vehicle (Tauber et al., 2018).[23] In the present work, we observed that adverse effects and patient discomfort was significantly higher with nanomicellar CAs 0.09%. These observations suggest that although the enhanced drug delivery due to nanomicelles CAs did better improve dry eye signs, the irritant nature of CAs perse led to adverse effects and patient discomfort.

The effect of CAs on goblet cell density in patients with dry eye disease remains controversial. In a non-obese diabetic (NOD) mice model (n=77) comprising seven groups of 11 mice each, Burade et al demonstrated that mice in the CAs ophthalmic solution 0.09% twice-daily group had significantly higher ($P<0.01$) goblet cell density compared with mice in the placebo and NOD diseased control groups after 60 days of treatment. Second, there was no significant difference in corneal staining and IL-1β levels with CsA 0.09% solution despite increase in tear volume. However, baseline goblet cell density was not measured in this study. The authors could not explain how goblet cell density increased after treatment with CAs. The present work was accomplished in a significantly larger number of subjects (77 versus 450) with no change from baseline goblet cell density in either treatment arms at 3 months (Burade et al., 2020).

CONCLUSION

In conclusion, higher strength CAs 0.09% significantly better improves corneal staining, tear production and tear film osmolarity but not goblet cell density and tear film stability as compared to CAs 0.05% in patients with dry eye disease.

LIMITATIONS

Limitations of the present study were absence of a control arm despite a randomized study design, a short study period as there were persistent dry eye symptoms after 3 months in both treatment arms and ocular surface markers of inflammation could not be evaluated due to cost constraints.

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