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ORIGINAL ARTICLE

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Randomized controlled study

Comparison of 20% autologous-serum eye drops with unpreserved hypromellose in the treatment of aqueous-tear-deficient dry-eye disease

ABSTRACT

Objective

This study evaluated the effectiveness of 20% autologous-serum eye drops versus unpreserved hypromellose in the treatment of patients with aqueous-tear-deficient dry-eye disease.

Methods

Patients fulfilling entry criteria were randomized to either 8 weeks of 20% autologous serum eye drops or 8 weeks of unpreserved hypromellose eyedrops. Changes from baseline at 1, 2, 4, and 8-week values of corneal and conjunctival staining with fluorescein and lissamine green, tear-break-up time (TBUT), Schirmer test (with anesthesia), and ocular-surface-disease index (OSDI) were measured. Statistical analyses were carried out using analysis of variance (ANOVA) and the Bonferroni-Holm adjustment.

Results

Thirty eyes (15 patients) in the autologous-serum group and 26 eyes (13 patients) in the unpreserved-hypromellose group completed the study. Corneal staining with lissamine green ($p = 0.05$) and conjunctival staining with fluorescein ($p = 0.04$) showed significant improvement in scores in the autologous-serum group compared to that of the unpreserved hypromellose group at 2 weeks. After 8 weeks of treatment, however, differences in staining scores, Schirmer test, and TBUT were not significant. The OSDI ($p = 0.002$) showed significantly greater improvement in the autologous-serum group than in the unpreserved hypromellose group.

Keywords: *Autologous-serum eye drops, Unpreserved hypromellose, Aqueous-deficiency dry-eye disease, Ocular-surface staining, Ocular-surface-disease index*

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Conclusions

Use of both 20% autologous-serum eye drops and unpreserved hypromellose is safe and effective as single treatment for aqueous-tear-deficient dry-eye disease. However, dry-eye condition improved earlier among patients in the autologous-serum group than those in the unpreserved hypromellose group, and provided greater functional improvement and symptomatic relief.

INTRODUCTION

Normal sight relies on a moist ocular surface. A sufficient quantity, quality, and normal composition of tears; normal lid closure; and regular blinking are needed to maintain this status. These factors control the maintenance of the preocular tear film, which protects the eye from drying. The tear film and ocular surface form a complex and stable system that may lose its equilibrium through numerous disturbing factors,[1] leading to ocular-surface desiccation and causing discomfort symptoms like dryness, burning, stinging, grittiness, irritation, and foreign-body sensation.[2]

Much research has been conducted around the world to gain a better understanding of dry-eye pathology and treatment. Dry eye is now thought to be a relentless cycle of ocular-surface irritation, inflammation, and immune activation that leads to decreased tear production through a negative feedback mechanism.[3, 4, 5]

This concept has led to the development of more targeted therapies as the focus of treatment shifts from symptomatic relief to addressing the underlying disease. Essential in the management of this disease is ocular-surface healing, which is vital to relieving irritation and disrupting this vicious cascade of events.

Artificial tears are still the most frequently used preparations for managing this condition and providing symptomatic relief. Currently, a wide range of commercial products is available,[6] from which may be selected an individual regimen for a particular patient that may either be a single agent or a combination.

In 1984, Fox et al. first reported the beneficial effects of autologous-serum application to dry eye in Sjogren's syndrome. The rationale for their observation was based on the fact that vitamins or growth factors present in tears, integral to ocular surface healing, are also present in serum.[7] The application of autologous serum offers an advantage over the simple use of artificial tears which lack such essential components. Moreover, autologous-serum drops are nonallergenic and their biomechanical and biochemical properties are similar to those of normal tears.[8]

Autologous-serum eye drops have been found in uncontrolled trials to benefit patients with severe dry eye, improving the ocular surface and reducing symptoms. [9-15] In these studies, however, varying methods of preparation and different serum concentrations—from 20% to 100%—have been used.

In a two-week prospective randomized case-control study, Kojima et al. compared the effect of autologous-serum eye drops with unpreserved artificial tears in the treatment of severe dry-eye disease.[16] Tear-break-up time (TBUT), vital staining, Schirmer test, and pain-analog scores were assessed after a washout period. These parameters showed significant improvement with autologous-serum eye drops. However, the relatively short duration of treatment limits its use in clarifying the continued efficacy and risks of prolonged autologous-serum application. Furthermore, a functional assessment of the severity of dry-eye disease was not performed.

We, therefore, compared the effects of autologous-serum eye drops and unpreserved hypromellose (Tears Naturale Free, Alcon Laboratories, Fort Worth, TX, USA) in the treatment of aqueous-tear-deficient dry-eye patients in an 8-week randomized controlled trial by evaluating TBUT, ocular-surface staining, and Schirmer test. Vision-related functional assessment of dry-eye severity using the ocular-surface-disease index (OSDI) was also performed.[17]

METHODOLOGY

We performed an eight-week investigator-masked, randomized, controlled trial at the University of the Philippines–Philippine General Hospital (UP–PGH). The research followed the tenets of the Declaration of Helsinki and informed consent was obtained from all the subjects after the nature and possible consequences of the study were explained. The Institutional Review Board approved the protocol and informed-consent form.

Sixty eyes of 30 patients with aqueous-tear-deficient dry eye and ocular-surface staining were enrolled into the study from January to June 2005. Subjects were recruited from the outpatient eye clinics of the UP–PGH Department of Ophthalmology and Visual Sciences. All patients suspected of having dry-eye disease underwent a complete evaluation including collection of demographic data, ocular, medical, surgical, and medication history; OSDI assessment; visual-acuity testing using the ETDRS acuity chart; intraocular-pressure measurement; funduscopy; slitlamp examination; TBUT; fluorescein (Fluo Strips, Contacare, Baroda, India) and lissamine-green (Lissamine Green Strips, Contacare, Baroda, India) corneal and conjunctival staining; and Schirmer test with anesthesia.

The inclusion and exclusion criteria limited the type of dry eye to symptomatic aqueous-tear-deficient dry eyes with ocular-surface staining. In the absence of a universally accepted classification for dry-eye disease or dysfunctional-tear syndrome or lacrimal keratoconjunctivitis, we did not specifically define the pathogenesis of the dry eye of the

study subjects. But based on the patients' characteristics, it can be seen that the great majority were age-related, possibly androgen-deficient, postmenopausal dry-eye sufferers. Excluded were patients with a history of ocular pathology other than error of refraction and cataract, contact-lens wear, glaucoma, ocular surgery or trauma affecting corneal sensation, severe ocular-surface disease (chemical burn, Stevens-Johnson Syndrome, ocular cicatricial pemphigoid, etc), herpes-simplex keratitis, active ocular allergy, active ocular infection and non-dry-eye-induced inflammation, severe blepharitis, lid abnormalities and lacrimal puncta malposition, use of any form of topical ophthalmic medications, use of chronic systemic medications that may affect dry-eye condition, or known hypersensitivity to hypromellose, as well as pregnant or nursing females.

Subjects who fulfilled all the inclusion criteria and signed the informed-consent form were assigned a study number in ascending order for documentation. Qualified subjects were randomly assigned by an independent research assistant to receive either 8 weeks of 20% autologous-serum eye drops or 8 weeks of unpreserved hypromellose (Actives: Duasorb water soluble polymeric system containing dextran 70 0.1% and hydroxypropyl methylcellulose 2910 0.3%) (Tears Naturale Free or TNF, Alcon Laboratories, Fort Worth, TX, USA) based on a predetermined list of random numbers. Those in the autologous-serum group were sent to the hospital's clinical chemistry laboratory for serum preparation.

The autologous-serum eye drops were prepared according to the protocol by Tsubota and associates.[7] A total of 30 ml of blood was secured by venipuncture into a sterile vial, allowed to stand for 60 minutes for clotting to occur and centrifuged for 5 minutes at 250xg (1,500 rpm). Serum was then carefully separated in a sterile manner and diluted by physiologic saline to 20%. Six-milliliter aliquots were transferred into sterile bottles with ultraviolet-light protection and properly labeled with the pertinent patient data and date of preparation.

Written instructions were given to all subjects to instill the assigned drops only 6 to 10 times daily in each eye. Subjects in the autologous-serum group were instructed to:

• use one bottle per week and surrender the empty bottle on follow-up,

• prevent the dropper tip of the bottle from getting in contact with any surface and replace the cap after each use, and

• keep the bottle being used in the refrigerator (or coolant bags provided) and those for succeeding weeks in the freezer to be thawed inside the refrigerator 24 hours before use. (If the patient did not have a freezer, they were stored in the hospital freezer until required.)

Subjects in the unpreserved-hypromellose (Tears Naturale Free or TNF) group were instructed to use as many ampules a day for a total of 6 to 10 times a day in each eye and discard all opened ampules at bedtime. All unused ampules were returned for inventory.

All subjects were asked to keep a daily record of the number of times eye drops were instilled and to return for evaluation at 1, 2, 4, and 8 weeks of treatment.

The examiners were masked to the treatment given to the patients. All questions regarding the medications were received by the independent research assistant and forwarded to the investigators if needed. On follow-up visits, assessment of adverse events, concomitant medications, concurrent procedures, and subjective tolerability upon instillation was done by the independent research assistant and the patients were reminded not to discuss their treatment with the investigators during ophthalmologic examination.

Outcome Measures

Efficacy. The objective signs monitored were TBUT, corneal and interpalpebral conjunctival staining with fluorescein and lissamine green, and Schirmer test with anesthesia, in that order. All these variables were evaluated at baseline and at each follow-up visit. For subjective measure, OSDI was evaluated at baseline and at 4 and 8 weeks.

TBUT was determined during the two-minute wait for corneal staining. The fluorescein strips were moistened with one drop of normal saline and applied onto the inferior palpebral conjunctiva, avoiding contact with the ocular surface. TBUT was taken within 30 seconds after instillation of fluorescein with a slitlamp set at diffuse illumination and 10X magnification using cobalt-blue light. Three consecutive TBUTs were obtained and recorded per eye.

TBUT was defined as the point in time when an area of fluorescein discontinuity is detected that includes the central part of the cornea and not just the periphery. Time until random-location tear break-up between blinks was measured only up to 10 seconds.

Corneal staining with fluorescein was evaluated within 30 seconds but less than 2 minutes from application with a slitlamp set at diffuse illumination and 16X magnification using cobalt-blue light.

The lissamine green strips were moistened with 2 drops of normal saline, agitated for 15 seconds, and then applied to the lower palpebral conjunctiva. Corneal and conjunctival staining with lissamine green were evaluated within 30 seconds but less than 2 minutes after instillation using a slitlamp set at diffuse illumination and 16X magnification with white light.

For corneal and conjunctival staining, 5 different areas of the cornea and 6 areas of the interpalpebral conjunctiva were graded based on the 5-point Oxford Scheme of

Staining (0 to 4) and scores averaged for the final staining grade. A negative change from baseline indicated an improvement.

The Schirmer test was performed 2 minutes after instillation of topical proparacaine (Alcaine, Alcon Laboratories, Fort Worth, TX, USA) using standard sterile tear-measurement strips (Tearstrip, Contacare, Baroda, India) kept in the lower conjunctival sac for 5 minutes under standard condition. A positive change from baseline indicated improvement.

The OSDI questionnaire was used to evaluate the impact of each patient's dry-eye disease on vision-related functioning. It consisted of 12 questions that were each rated from 0 (none of the time) to 4 (all of the time). The total OSDI score was then calculated based on the following formula: OSDI = (sum of scores for all questions answered) X 100/ (total number of questions answered) X 4.[17] Higher scores reflected greater disability and a negative change from baseline indicated improvement.

Tolerability. Subjective evaluation of level of comfort (tolerability) upon instillation was also assessed, from 1 (very comfortable) to 4 (very uncomfortable). Study product usage (i.e., number of times subjects instill product daily) was also monitored at each follow-up visit.

Safety. The primary safety variable monitored was the occurrence of adverse events. The severity of each adverse event observed was rated as mild (awareness of sign or symptom but easily tolerated) to severe (incapacitating with inability to perform usual routine). The relationship to the study medication was assessed by the evaluators and appropriate action taken. Other safety variables monitored were best-corrected visual acuity, applanation tonometry, biomicroscopy, and funduscopy. Safety variables were evaluated at all visits except for applanation tonometry and funduscopy that were done at baseline and exit visits.

Statistical analysis

Change in variables from baseline to week 8 was the primary analysis. The change from baseline was calculated as follow-up minus baseline so that a negative change indicated improvement. All numerical continuous data were summarized using descriptive statistics (percentage, frequency distribution, mean, and median). Mann-Whitney U test for the comparison of ranks between 2 independent samples were used to analyze continuous numerical variables. Discrete categorical variables of the two groups were compared using chi-square.

To test for changes in independent variables that are continuous and numeric (corneal staining grade), repeated measures analysis of variance was employed. To compare mean change in ranks at baseline and at 8 weeks of observation, two-way analysis of variance by Friedman was utilized. Posthoc analysis was performed using the Bonferroni-Holm adjustment to determine which comparisons were significantly different.

All statistics were carried out with the Statistical Package for the Social Sciences (SPSS Version 10) and Statistics version 1998. Hypothesis testing was carried out at .05 level of significance to achieve a type II error of at least 0.2 and study power of at least 80%.

RESULTS

A total of 30 subjects were enrolled into the study. Two (2) subjects in the unpreserved-hypromellose group (TNF) dropped out of the study for personal reasons unrelated to the treatment. The outcomes from 56 eyes were included in the final analysis. The youngest patient in this study was 18 years old and the oldest was 78 years

Table 1. **Baseline characteristics of study subjects (N = 28).**

Characteristic	Autologous Serum n = 15	Unpreserved Hypromellose n = 13	*P*[a]
Age			
Range	23 to 74	18 to 78	
Mean ± SD	57 ± 15	52 ± 19	0.62[b]
Median	57	51	
Sex			
Male	3	4	0.51[c]
Female	12	11	
OSDI			
Range	15 to 53	15 to 52	
Mean ± SD	28.2 ± 9	26.5 ± 13	0.29[b]
Median	27.5	22.5	
Discomfort scale			
Range	3 to 14	1 to 13	0.40[b]
Mean ± SD	9 ± 3	8 ± 4	
Median	9	9	
Tear-break-up time (seconds)			
Range	3 to 9.3	2 to 9.3	0.46[b]
Mean ± SD	5.8 ± 2	6.1 ± 2	
Median	5.5	6.3	
Corneal staining			
Fluorescein			
Range	0 to 4	0 to 1.2	
Mean (units) ± SD	0.48 ± 1	0.35 ± 0.37	0.16[b]
Lissamine green			
Range	0 to 3.6	0 to 1	
Mean (units) ± SD	0.44 ± 0.9	0.3 ± 0.3	0.25[b]
Conjunctival staining			
Fluorescein			
Range	0.3 to 4	0.3 to 1.3	
Mean (units) ± SD	1 ± 0.86	0.65 ± 0.33	0.10[b]
Lissamine green			
Range	0.3 to 4	0.3 to 1.5	
Mean (units) ± SD	1.1 ± 0.89	0.77 ± 0.4	0.25[b]
Schirmer test			
Range	0 to 10	2 to 10	
Mean ± SD	5 ± 3	6 ± 3	0.17[b]

[a]Significant difference if p-value <.05, computed using SPSS version10
[b]Computed using Mann-Whitney U Test for ranked data, SPSS version 10
[c]Computed using Chi-square, SPSS version 10

old. Mean age was 54 ± 16 years and the median was 55 years. Females outnumbered males 3 to 1 (23 to 7). The baseline characteristics and tests of homogeneity of the sample are summarized in Table 1.

There was no statistically significant difference in age ($p = 0.62$), sex distribution ($p = 0.51$), baseline OSDI ($p = 0.29$) and baseline TBUT ($p = 0.46$) between the 2 groups. There was no statistically significant difference in baseline corneal and conjunctival staining with both fluorescein and lissamine green and Schirmer test results for all eyes examined ($p > .05$ for all).

Corneal fluorescein staining. Results showed a nonstatistically significant drop in the mean corneal-staining scores for each group ($p = 0.35$) with a more constant slope seen with autologous serum than TNF (Figure 1). In the TNF arm, scores showed a slight rise at 4 and 8 weeks in contrast with the autologous-serum arm which exhibited a continuously decreasing score up to 8 weeks. Although autologous serum caused a greater drop in mean staining scores from baseline, the overall drop in mean corneal staining scores in the serum group was not significantly different from the TNF group ($p = 0.29$).

Corneal lissamine green staining. Although there was a decreasing trend in the mean staining scores using lissamine green, no significant change from baseline was noted for each group ($p = 0.84$) (Figure 2). A difference of 0.2 score units from baseline was noted after 2 weeks of treatment. This difference in scores for both autologous serum ($p < 0.001$) and TNF ($p = 0.003$) were statistically significant. However, the overall drop in mean corneal-staining scores between the two groups did not significantly differ across the weeks of observation ($p = 0.35$) (Figure 2).

Conjunctival fluorescein staining. A steep drop in mean staining scores from baseline to 2 weeks was seen with the serum group, which gradually plateaued at the 4th and 8th weeks while a nonsloping trend was noted with the TNF group. The change in mean staining scores was significantly different in the observation periods within each group ($p = 0.001$) (Figure3). Bonferroni-Holm adjustment showed that the difference in staining in the first week was significant for both serum and unpreserved hypromellose. Although the serum group had a greater drop in mean scores during the initial phases of treatment, there was a convergence of the two lines at the 3rd week. This difference was not statistically significant ($p = 0.29$).

Conjunctival lissamine green staining. A steep drop in mean conjunctival-staining scores from baseline to 4 weeks was noted in the serum group while a nonsloping trend was seen in the TNF group which were statistically significant ($p = 0.04$) (Figure 4). Bonferroni-Holm adjustment showed that a significant difference in staining was seen during the first and second weeks of both

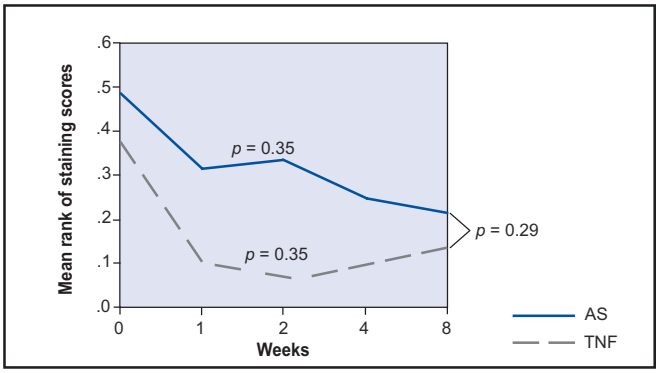

Figure 1. Corneal fluorescein staining across 8 weeks of observation.

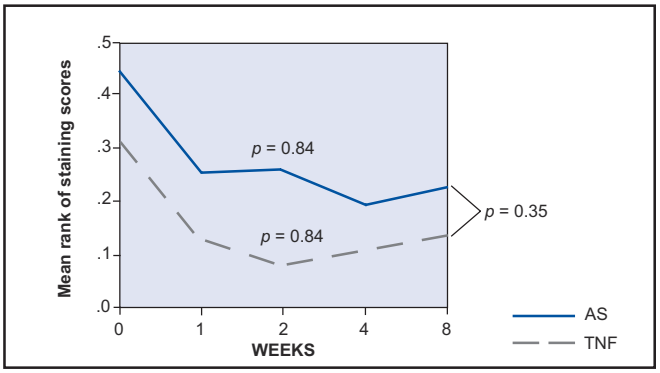

Figure 2. Corneal lissamine-green staining across 8 weeks of observation.

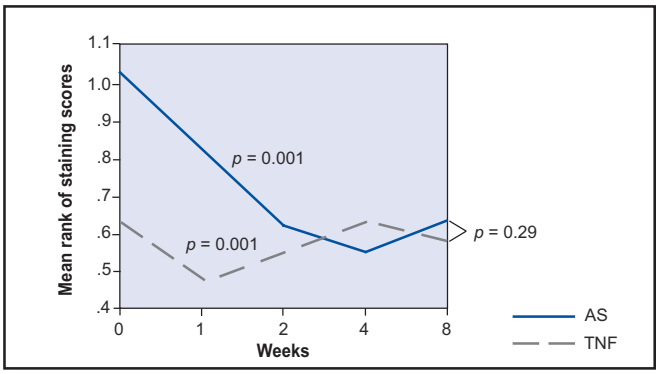

Figure 3. Conjunctival fluorescein staining across 8 weeks of observation.

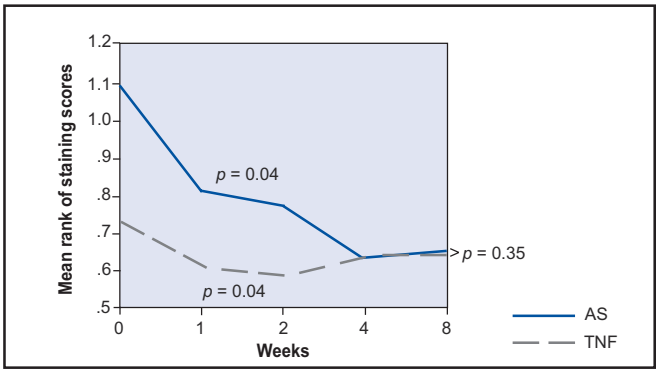

Figure 4. Conjunctival lissamine-green staining across 8 weeks of observation.

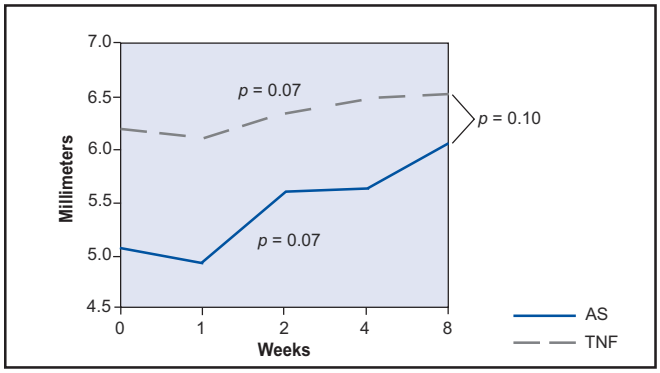

Figure 5. Schirmer test at baseline, 1, 2, 4, and 8 weeks of treatment.

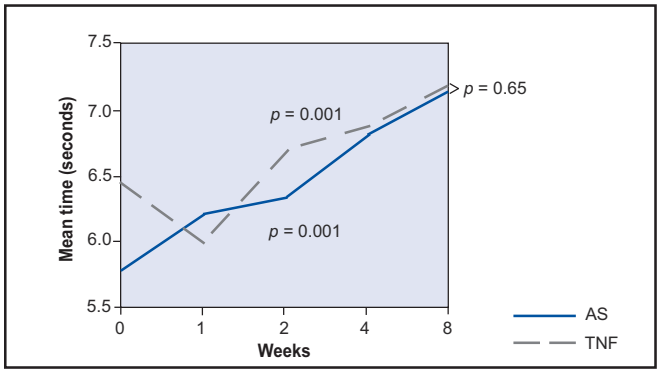

Figure 6. Mean tear-breakup time at baseline, 1, 2, 4, and 8 weeks of treatment.

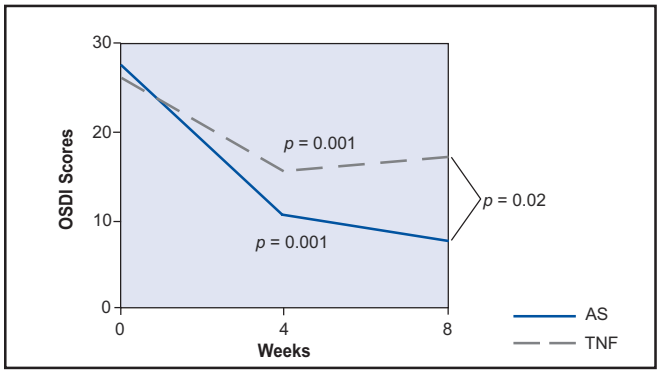

Figure 7. Mean ranks of OSDI scores at baseline, 4, and 8 weeks of treatment.

treatments. No significant difference was seen between the serum and TNF groups across the periods of observation ($p = 0.35$).

Schirmer test. Results from baseline to 2 weeks were not significantly different within each group ($p = .07$). There was no significant difference between groups in terms of the degree of change across the eight-week period ($p=0.10$) (Figure 5).

Tear-breakup time. A gradual increase in TBUT was noted from baseline in the autologous-serum group (mean 5.8 to 7.1 seconds at 8 weeks, mean difference of 1.3 seconds). Comparing baseline and 8 weeks, the difference was statistically significant ($p = 0.001$). An initial drop was seen from baseline to the first week of treatment in the TNF group, which gradually increased thereafter (mean 6.1 seconds to 7.2 seconds, mean difference of 1.1 seconds) (Figure 6). Comparing baseline and 8 weeks, the difference in time was statistically significant ($p = 0.001$). Bonferroni-Holm adjustment showed that a significant difference was noted after the second week only. However, comparing autologous serum and TNF, no significant difference in TBUT was seen after 8 weeks of treatment ($p = 0.65$).

Ocular-surface-disease index. Mean ranks of OSDI scores were statistically lower from baseline after 4 and 8 weeks of treatment with autologous serum ($p = 0.001$) and TNF ($p = 0.001$), which was confirmed by a subsequent Bonferroni-Holm adjustment. At the eighth week of treatment, mean ranks of OSDI scores were significantly lower in the autologous-serum group compared with the TNF group (mean rank difference = 7.2 points, $p = 0.02$) (Figure 7).

Tolerability profile. We assessed the tolerability of the two interventions based on a five-point index of comfort at 2, 4, and 8 weeks of treatment. Most patients were comfortable with use of either autologous serum or unpreserved hypromellose. One patient in the unpreserved-hypromellose group complained of slight discomfort that occurred only during the first week of use.

Frequency of instillation of eyedrop. There was no significant change in the average number of times the eye drops were instilled across time whether autologous serum or TNF ($p = 0.46$). Although autologous sera were instilled more during the first to the fourth week, this was not statistically different from TNF instillation.

Adverse events. Only one patient complained of mild itching in the TNF group on the first follow-up visit. No other adverse events were reported.

DISCUSSION

Several uncontrolled clinical trials have shown the beneficial effects of autologous-serum eye drops in the treatment of severe dry-eye disease, Sjogrens syndrome,[7] recurrent corneal erosions,[10, 12, 15] superior limbic keratoconjunctivitis (SLK),[13] neurotrophic keratopathy,[14] and graft-versus-host disease. These have laid the groundwork for studies comparing autologous serum with the most widely used conventional therapy for dry-eye disease—artificial-tears eye drops.

In a two-month controlled trial of 12 dry-eye patients comparing autologous-serum eye drops in one eye with unpreserved normal saline in the fellow eye, Tananuvat and associates[2] reported a nonsignificant trend toward improvement of ocular-surface staining and tear stability in both eyes. One source of bias, however, is that most patients in that study had undergone punctal occlusion, which might have caused overvaluation of the effects of

artificial tears. Our results support their findings even as none of our patients had received punctal occlusion.

In a randomized controlled crossover trial comparing autologous serum with different conventional therapies in the treatment of severe ocular-surface disorder, Noble and associates reported improvement of impression cytology parameters.[9] However, no differences were found for vital staining, Schirmer test, or tear-clearance test. The crossover design of the study confirmed that these improvements were due to serum drops as the effect was reversed when the treatment was reversed. A presumption then was that the beneficial effects were due to the presence of essential tear factors.

A recent prospective randomized case-control study of 37 eyes of 20 patients by Kojima and associates[16] assigned 2 groups of severe dry-eye disease patients to either 2 weeks of autologous-serum eye drops or unpreserved artificial tears after a two-week washout period with unpreserved saline drops. Use of autologous serum was associated with a statistically significant improvement in double-vital-staining scores, TBUT, and pain-symptom scores. This is consistent with our findings during the first 2 weeks of treatment where corneal- and conjunctival-staining scores with fluorescein and lissamine green were lower in the autologous-serum group. At the end of the eight-week trial, however, we found insignificant differences in ocular-surface-staining scores, Schirmer test and TBUT scores in patients treated with autologous serum compared with those treated with unpreserved hypromellose. This suggests a plateau in the effect of autologous serum beyond two weeks, which was not evident in the study of Kojima et al. because of their short follow-up period.

The OSDI, however, showed significant improvement in those treated with autologous serum than those treated with artificial tears, consistent with the findings of previous studies of superior subjective dry-eye-symptom relief. The use of the OSDI enabled us to evaluate the impact on the patients' vision-related functioning, rather than just a single symptom of pain or discomfort.

The faster onset of relief of signs and symptoms from autologous serum would be of great benefit to the patient notwithstanding the rigors of its handling and preparation. The effectiveness of "induction" treatment with autologous serum for 2 to 3 weeks followed by "maintenance" with artificial tears, compared with autologous-serum monotherapy or artificial-tears monotherapy, could be the subject of future investigations.

It would also be interesting to find out the prolonged effects of autologous serum in the treatment of dry-eye disease and the effects of different concentrations of autologous-serum eye drops. A recent study by Liu et al. reported the optimal clotting time, centrifugation forces, type of diluent and dilutions that will increase the concentration of growth factors, fibronectin, and vitamins in autologous-serum eye drops and improve its epitheliotrophic capacity which they tested in a cell-culture model.[18] Future randomized clinical trials should be done to validate the clinical efficacy and safety of their protocol.

A limitation of this study is that our subjects were not masked, which might have affected their response to the OSDI questions. Moreover, we cannot discount the fact that the cold temperature of the serum drops might have affected their OSDI scores as well. However, we feel that our study design enabled us to simulate how these eye drops are used in actual clinical setting, which is in line with the objectives of this study.

In summary, we found that 20% autologous-serum eye drops and unpreserved hypromellose are safe and effective as sole treatments for aqueous-tear-deficient dry-eye disease. However, patients' dry-eye condition improved earlier in the autologous-serum group compared with the unpreserved-hypromellose group and provided better functional improvement and symptomatic relief.

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