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Tear Function and Ocular Surface Findings in Premature and Term Babies

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Objective: To describe the ocular surface and tear function findings in premature and term babies.

Design: Prospective, case–control study.

Participants: Forty-eight eyes of 24 premature babies seen at the Department of Ophthalmology of Uludag University School of Medicine, Bursa, Turkey, from March 2002 through September 2002 and 50 eyes of 25 healthy term babies were studied.

Intervention: The subjects underwent routine ophthalmic examinations; corneal sensitivity measurements; Schirmer test with anesthesia, with and without nasal stimulation; primary Jones test; fluorescein staining of the ocular surface; and conjunctival impression cytology.

Main Outcome Measures: Premature and term babies were compared for corneal sensitivity, lacrimal drainage system patency, tear function and ocular surface staining parameters, goblet cell density, and squamous metaplasia grade. The relation of these parameters to the status of the ocular surface was also investigated.

Results: Mean corneal sensitivity scores were 45±5.0 mm and 55±4.5 mm in the premature and term babies, respectively ($P<0.001$). Premature babies had a mean corneal fluorescein staining score of 1.5±0.25 points, compared with 0.22±0.28 points in the term babies ($P<0.001$). The mean Schirmer test scores without and with stimulation were 1.5±2.5 mm and 4.15±2.5 mm in the premature babies, respectively, compared with 15±3.5 mm and 18.75±4.5 mm in the term babies. The intragroup and intergroup Schirmer test scores were statistically significant ($P<0.001$). The primary Jones test was positive in 20.8% of the eyes in the premature babies, whereas it was positive in 84% of eyes in the term babies. The premature babies with positive primary Jones test results all had corneal epithelial defects or severe superficial punctuate keratopathy. Mean conjunctival impression cytology squamous metaplasia scores were 1.86±1.2 in the premature babies and 0.86±0.47 in the term babies ($P<0.001$). Mean goblet cell densities were 393±484 cells/mm^2 and 739±503 cells/mm^2 in the premature and term babies, respectively ($P<0.001$).

Conclusion: Decreased corneal sensitivity, reduced tearing, and lacrimal drainage patency are important determinants of ocular surface disease in premature infants. Premature newborns with low Schirmer test scores and a patent lacrimal system may experience corneal and conjunctival epithelial problems and should be carefully checked for the presence of dry eye complications. *Ophthalmology 2004;111:901–905 © 2004 by the American Academy of Ophthalmology.*

Although much has been learned about the ophthalmic retinal complications of prematurity during recent years, the impact of prematurity on the status of the ocular surface and the associated alterations still need to be clarified. How prematurity-induced ocular surface alterations relate to tear function and lacrimal system patency is also controversial. An increased understanding of the ocular surface status in premature and term infants, including alterations of the tear film, lacrimal drainage patency, and the conjunctival cells, is highly essential. Therefore, we performed corneal sensitivity measurements; Schirmer tests with anesthesia, with and without nasal stimulation; primary Jones test; fluores-

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cein staining of the ocular surface; and conjunctival impression cytologic analysis to analyze the effect of prematurity on the ocular surface health, and also compared the results with those of term control infants.

Materials and Methods

Subjects and Examinations

Forty-eight eyes of 24 premature babies (13 male and 11 female) born between 24 and 35 gestational weeks (mean: 30.5) as well as 50 eyes of 25 term babies born between 37 and 41 gestational weeks (mean: 39.4; 13 male and 12 female) were recruited from the Department of Neonatology of Uludag University Hospital from March 2002 through September 2002. Both groups were similar regarding gender characteristics. Fundus examinations consisted of direct and indirect ophthalmoscopy for retinopathy of prematurity (ROP) screening in the premature babies. The eyes of all premature infants born at less than 35 weeks and weighing less than 1500 g were screened for ROP. The premature and term infants underwent ocular surface examinations 4 weeks after birth, including fluorescein corneal staining, corneal sensitivity measurements, Schirmer tests with and without nasal stimulation, primary Jones test, and conjunctival impression cytology. All examinations were performed before the fundus examinations by the same researcher (HK). Informed consent from the parents as well as permission from the Ethics Committee of the Uludag University Faculty of Medicine were obtained. These examinations were also carried out on those mothers who requested that the procedures should be performed on themselves first before allowing them on their babies. No baby was being treated with topical eye medications at the time of impression cytology. Infants who were scheduled for an ROP screening received topical 0.5% cyclopentolate eyedrops 3 to 4 hours before the ocular surface examinations. Infants with neurologic disorders, intraventricular hemorrhage, hydrocephalus, chromosomal abnormalities, convulsions, labor asphyxia, Apgar score of <8, or a history of any ocular or systemic disease that would alter the ocular surface assessments were excluded. Birth weight, head circumference, medical and neurologic status, including doll's eye reflex, pupillary light reflex, and oculovestibular reflex, were determined and recorded for each baby by the same researcher (NK) from the Department of Neonatology. The researchers also took the task of screening the infants every day for 4 weeks after birth for their daily activities, which were recorded to determine when the infants would be most alert and to guide the authors to schedule their ocular surface examinations in accordance with each infant's alertness hours.

Fluorescein staining of the cornea was initially performed and scored as described elsewhere.[1] Fluorescein staining scores ranged between 0 and 3 points. A score higher than 1 point was regarded as abnormal. Fluorescein staining was carried out by instillation of 2% fluorescein eyedrops (Alcon Inc., Istanbul, Turkey).

Measurement of corneal sensitivity was performed using a Cochet–Bonnet esthesiometer (Handaya, Tokyo, Japan). One examiner gently held the baby's head from behind, and a second examiner carried out the measurement. Both examiners assessed the presence of tactile corneal reflex in the infant, and a response was considered present when both examiners agreed. If the baby lost attention and fell asleep, or if the response was considered doubtful, the corneal sensitivity testing was terminated. However, the researchers made extra efforts to visit the same infant several times within the same day to carry out the corneal sensitivity measurement whenever they were paged and informed by the Neonatology Department staff that the infant was awake and alert again. The measurements were begun with the nylon filament fully extended. The tip of the nylon filament was applied perpendicularly to the surface of the cornea, making certain not to touch the eyelashes, and was pushed until the fiber's first visible bending. The length of the fiber was gradually decreased until a blink reflex was observed and confirmed by both examiners. The length was recorded in millimeters. Measurements were taken from the central cornea 3 times, and the mean of the measurements was recorded as the corneal sensitivity reading of that eye. A corneal sensitivity measurement of less than 50 mm was regarded as low corneal sensitivity in this study.[2,3]

For further evaluation of tears, the standard Schirmer test with topical anesthesia (0.4% oxybuprocaine chloride) was performed. The standardized strips of filter paper (Alcon Inc., Fort Worth, TX) were placed in the lateral canthus away from the cornea and left in place for 5 minutes. Readings were reported in millimeters of wetting for 5 minutes. A reading of less than 5 mm was referred to as dry eye, according to Japanese Dry Eye Diagnostic Criteria.[4] A Schirmer test with nasal stimulation was performed several minutes after the first testing by inserting a cotton swab into the baby's nasal cavity. A series of sneezes was regarded as a positive response indicating adequate nasal stimulation. This repeat Schirmer test was conducted for 5 minutes while the cotton swab was kept in place.

Primary Jones dye testing was performed to assess the patency of the lacrimal drainage system. Fluorescein recovery from the nose as determined by yellow discoloration of the cotton swabs was regarded as a positive test and indicated the patency of the drainage system. A secondary (irrigation) Jones test to evaluate the mechanism of obstruction was not carried out because a permit was not granted by the ethics committee for this examination. Examinations to be performed were allowed once on the day of the initial examination and not in consecutive weeks.

The impression cytology specimens were obtained after administration of topical anesthesia with 0.4% oxybuprocaine. Strips of cellulose acetate filter paper (HAWP 304, Millipore Corp., Bedford, MA) that were soaked in distilled water for a few hours and dried at room temperature were applied on the lower nasal bulbar conjunctiva adjacent to the corneal limbus, pressed gently by a glass rod, and then removed. The specimens were then fixed with formaldehyde, stained with periodic acid–Schiff, dehydrated in ascending grades of ethanol and then with xylol, and finally coverslipped. The quantitative studies of conjunctival goblet cells and squamous metaplasia of conjunctival epithelial cells were conducted by taking photographs using a calibrated grid under a light microscope at a magnification of ×400. We photographed 5 overlapping areas of each sample selected at random and averaged the outcomes for a single sample score. The goblet cell densities were reported as cells per square millimeter with standard deviations. The specimens were also assigned a grade of conjunctival epithelial squamous metaplasia according to Nelson's grading scheme.[5] All specimens were evaluated by the same researcher (MD), who was masked as to whether the specimens came from premature or term infants.

Statistical Analysis

Data were processed using StatView.[6] The Mann–Whitney test was used for the analyses of nonparametric values. A probability level of <1% was considered statistically significant. Analyses of categorized data were performed using the chi-square test, with the probability level set at 1% for statistical significance.

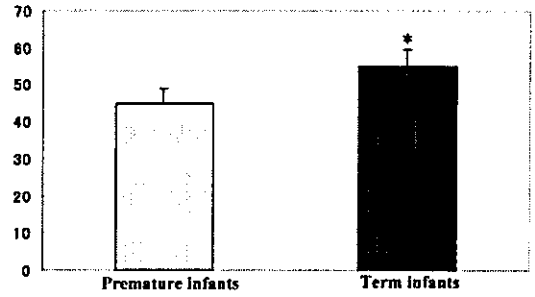

Figure 1. Corneal sensitivity changes (mm) in preterm and mature infants. *Mann–Whitney test, $P<0.001$.

Results

Clinical Features

There were no gender-related statistical differences between preterm and term infants. The average birth weight in premature infants was 1147±260 g (ranging from 628 to 1500 g), compared with 3262±720 g (ranging from 2050 to 3650 g) in term infants, and the difference was statistically significant ($P<0.001$). Neurologic evaluation of all premature and term infants, including doll's eye reflex, oculovestibular reflex, and pupillary light reflex, was normal. Alertness monitoring revealed that babies remained alert during, just before, and for 3 to 5 minutes after bottle feeding. Corneal sensitivity testing was completed successfully in 86% of the first visits. Revisits within the same day allowed quantification of corneal sensitivity in all remaining eyes.

Corneal Sensitivity

Corneal sensitivity could be quantified in all eyes. Forty-two eyes (87.5%) of the premature infants, but no term babies' eyes, had low corneal sensitivity. The mean corneal sensitivity scores were 45±5.0 mm and 55±4.5 mm in the premature and term babies, respectively. This difference was statistically significant ($P<0.001$), as shown in Figure 1.

Tear Function Parameters

All eyes of the preterm infants, but no term infants' eyes, had a fluorescein staining score exceeding 1 point. Premature babies had a mean corneal fluorescein staining score of 1.5±0.25 points, compared with 0.22±0.28 points in the term babies, as shown in Figure 2 ($P<0.001$). The Schirmer test without stimulation revealed that all premature babies' eyes had aqueous deficiency. None of the term infants had Schirmer test values less than 5 mm.

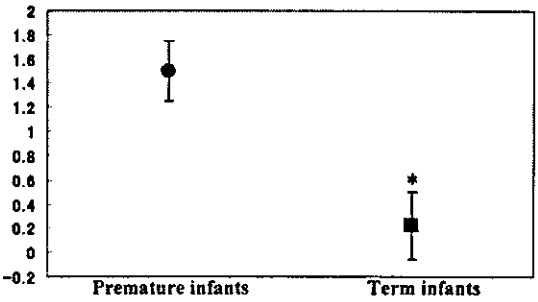

Figure 2. Fluorescein staining scores in preterm and mature infants. *Mann–Whitney test, $P<0.001$.

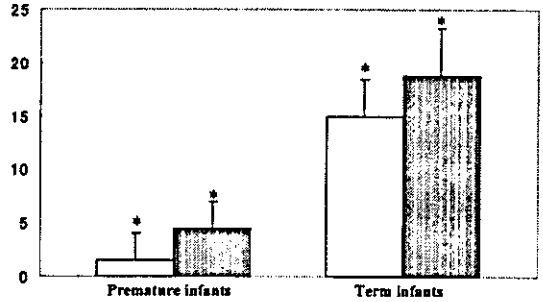

Figure 3. Schirmer test changes (mm) in preterm and mature infants. Schirmer test without nasal stimulation (white) and Schirmer test with nasal stimulation (grey). *Mann–Whitney test, $P<0.001$.

The Schirmer test with nasal stimulation showed the presence of reflex tears in all eyes of the premature and term infants. The mean Schirmer test scores without and with stimulation were 1.5±2.5 mm and 4.15±2.5 mm in the premature babies, respectively, compared with 15.0±3.5 mm and 18.75±4.5 mm in the term babies (Fig 3). The intragroup and intergroup Schirmer test scores were statistically significant ($P<0.001$). The primary Jones test was positive in 20.8% of the eyes in the premature infants, whereas it was positive in 42 eyes (84%) in the term babies (chi-square test, $P<0.001$). The eyes of the premature babies with positive primary Jones test results all had corneal epithelial defects.

Impression Cytology

Conjunctival imprints from the infants contained conjunctival epithelial cells, a variable amount of goblet cells, and mucin. Squamous metaplasia and the goblet cell density of each specimen were graded and calculated as described in "Materials and Methods." The mean conjunctival impression cytology squamous metaplasia score was 1.86±1.2 in the premature infants, compared with 0.86±0.47 in the term babies, as shown in Table 1 ($P<0.001$). The mean goblet cell densities were 393±484 cells/mm^2 and 739±503 cells/mm^2 in the premature and term babies, respectively ($P<0.001$).

Discussion

Previous studies have shown that full-term newborns produce tears normally and that preterm infants also have the function to secrete tears to a certain extent.[7,8] Two recent reports demonstrated reduced reflex and basal tear secretion in preterm infants.[9,10] The presence of decreased tactile corneal reflex in full-term infants[11] and abnormal conjunctival impression cytology in premature infants has also been reported.[12] How these disorders affect ocular surface health

Table 1. Impression Cytology Changes in Premature and Term Infants

	Premature Infants	Term Infants
Squamous metaplasia grade	1.86 ± 1.2*	0.86 ± 0.47
Goblet cell density (cells/mm^2)	393 ± 484*	739 ± 503

*$P < 0.001$, Mann–Whitney test.

and relate to lacrimal patency is still controversial. In this study, we performed corneal sensitivity measurements, corneal fluorescein staining, Schirmer tests with and without nasal stimulation, primary Jones test, and conjunctival impression cytology in premature infants to analyze the relation between the ocular surface status and these examinations, and also compared the results with those of full-term infants.

A PubMed database search revealed that corneal sensitivity has not been quantified in premature and term babies due to problems of alertness. It has been shown that tactile corneal reflex is positive in 80% of mature babies 4 to 8 weeks old.[10] We believe that our strategy of monitoring the babies for 1 month after birth to determine the hours during which they were awake and alert not only allowed a certain degree of neurologic maturation, but also helped us greatly in our attempts to measure the corneal sensitivity. We could show that 87.5% of the eyes of the premature infants had low corneal sensitivity and that all term babies had normal corneal sensitivity. A normal corneal sensitivity is known to provide trophic effects on the ocular surface, maintaining the epithelial milieu and functions.[13] We have shown in our previous studies that there should be a threshold corneal sensitivity value below which the ocular surface epithelium starts to disintegrate.[14] Indeed, 84% of the eyes of the preterm infants had a corneal fluorescein staining score exceeding 1 point. The increase of fluorescein staining scores not only might be due to decreased corneal sensitivity, but also might have been secondary to abnormalities in tear secretion. Therefore, we carried out Schirmer tests with and without nasal stimulation. Indeed, in accordance with previous studies, we found decreased basal and reflex tearing in the premature infants, but basal and reflex tearing reached normal values in the term babies. Differentiation of basal and reflex tearing in infants may be helpful in considering the diagnosis of certain diseases such as familial dysautonomia and congenital alacrima.[9] The presence of reflex tearing in all premature and term infants implied that the ocular surface had a natural defense mechanism when stressed with external/internal stimuli. We attributed the absence of dry eye–related complications in most of the premature babies to the presence of reflex tearing mechanisms. However, 20.8% of the eyes in the premature infants still had corneal epithelial defects or severe superficial punctate keratopathy. With the belief that these findings may be related to lacrimal patency, we carried out primary Jones testing. We found that 79.2% of the eyes in the premature babies had lacrimal obstruction, and that the rest of the eyes with patent lacrimal drainage system had epithelial defects or severe superficial punctate keratopathy. Unfortunately, we could not delineate the cause of lacrimal obstruction in this study, whether it was due to physiologic pump failure or to pathologic obstruction. Yet, lacrimal obstruction is known to be mostly physiologic in newborn infants, with attainment of patency within a few months after birth.[15] We believe that lacrimal obstruction in premature babies provides protection of the ocular surface by retention of available tears, thus compensating for decreased tearing. The presence of a very thick lipid layer in the tear film of premature and term infants with a prolonged noninvasive tear breakup time has been reported recently and may be another explanation for the absence of dry eye–related complications in most of the premature babies.[16]

We also carried out conjunctival impression cytologic analysis to check out the effects of decreased corneal sensitivity and reduced tearing on the general health of the ocular surface. Impression cytology provided evidence that prominent squamous metaplasia and goblet cell loss existed in premature babies, as opposed to term infants. Decreased goblet cell density is known to reflect ocular surface disease.[17] Reduction of goblet cell numbers may compound the tearing problems in infants by increasing tear film instability as a result of decreased mucin production, because goblet cells are the major source of mucin in tears, which is responsible for tear film stability.[18] Although we had a noteworthy observation that mucin pickup by the filter papers in the premature babies was almost nonexistent, and that the term infants had abundant mucin pickup, we could not answer whether a mucin deficiency state, in addition to aqueous deficiency, also existed in the premature infants because we did not measure the mucin content of tears this time. Concurrent involvement of conjunctival and corneal epithelial surfaces in the premature infants, as evidenced by prominent squamous metaplasia, markedly decreased goblet cell population, and corneal epithelial defects or superficial punctate keratopathy, may be viewed as an ocular surface disease related to primary or secondary events. The mechanisms of these ocular surface changes are still not clear, but a decrease of trophic effects of trigeminal sensory nerves on the conjunctiva and cornea as well as aqueous deficiency may be responsible. This study fell short of providing information about the effects of other possible factors on the ocular surface status in newborns, such as the role of blinking and Bell's phenomenon. It is our clinical impression that babies blink, but infrequently, which remains to be proved by further studies. We also could not provide answers about the timewise development of corneal sensitivity, tear functions, and cytologic ocular surface health parameters on consecutive weeks and when these parameters start attaining normal values. It is our assumption that tear quantity starts to normalize after 35 weeks when the differences in lacrimal drainage patency also start to lose their importance, as far as the ocular surface epithelium is concerned. Studies clarifying these issues would be very interesting.

One important implication of this study is on the current ophthalmic prematurity screening protocols, which mainly concentrate on the retinal disease. All ocular surface examinations in this study were performed before the fundus examinations. We came to understand that a simple corneal fluorescein staining conducted before screening helped in identifying those premature babies with ocular surface problems who were in need of supplemental lubrication. Most ophthalmologists carrying out screening for ROP are well aware of the fact that fundus visibility decreases due to corneal drying in premature babies and that frequent instillations of artificial tears are required during the examinations. We remind ophthalmic medical staff of the importance of ocular surface lubrication during fundus examinations in premature infants. Schirmer and primary

Jones tests in conjunction with fluorescein staining helped us to identify those premature babies who needed artificial tears until after 37 weeks. The findings of this study also justify avoidance of frequent instillations of topical medications with preservatives in preterm infants, due to preservative toxicity.[19,20] Decreased basal and reflex tear secretion in premature infants can limit the dilution of topical medication (especially in those infants with lacrimal obstruction), allowing the preservatives of the eyedrops in the tear film to become more concentrated than in older infants. Conversely, premature infants with a patent lacrimal drainage system may experience exaggerated effects due to absorption of the concentrated drug and their overall smaller body masses.[21] We advise simple external pressure over the punctal area for a few minutes after topical eyedrop instillations in premature infants to prevent possible systemic side effects.

Although premature infants with systemic and neurologic disease states were excluded from the current study, such infants may represent a special group whose members' ocular surface may be in jeopardy from additional stress due to the underlying disease. The ocular surface and tear functions of such babies remain to be determined.

In conclusion, our data suggest that decreased corneal sensitivity, reduced tearing, and lacrimal drainage patency are important determinants of the ocular surface disease in prematurity. Premature newborns with low Schirmer test scores and a patent lacrimal system may experience corneal and conjunctival epithelial problems and should be carefully checked for the presence of dry eye complications.

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Punctal Occlusion in the Management of Chronic Stevens–Johnson Syndrome

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Purpose: To evaluate the effect of lacrimal punctal occlusion in the management of the ocular surface disease in chronic Stevens–Johnson syndrome.

Design: Retrospective noncomparative case series.

Participants: Thirty-one eyes of 18 patients with chronic Stevens–Johnson syndrome seen at the Department of Ophthalmology of the Tokyo Dental College were studied.

Intervention: Patients' lacrimal puncta were occluded by cauterization or with punctal plugs.

Main Outcome Measures: The effect of lacrimal punctal occlusion was evaluated by changes in subjective symptoms, best-corrected visual acuity (VA), Schirmer test, tear clearance test, and ocular surface double vital staining before and after punctal occlusion.

Results: After lacrimal punctal occlusion, symptomatic improvement was observed in 19 eyes (61.3%). The mean logarithmic VA showed significant improvement from -0.64 ± 0.87 to -0.52 ± 0.86 ($P<0.05$). The Schirmer I test results before punctal occlusion yielded a mean score of 7.4 ± 8.7 mm, which changed to 10.2 ± 8.3 mm after punctal occlusion. The mean preocclusion and postocclusion tear clearance values were 7.5 ± 6.6 times and 4.9 ± 4.8 times, respectively. The Rose Bengal staining score decreased from 4.7 ± 2.8 to 2.7 ± 2.2 points ($P<0.05$), and the fluorescein staining score decreased from 5.0 ± 2.3 to 2.2 ± 2.5 points ($P<0.05$), respectively.

Conclusions: Concurrent improvements in subjective symptoms, vital staining scores, and VA point to the favorable effects of lacrimal punctal occlusion for the ocular surface health in chronic Stevens–Johnson syndrome. *Ophthalmology 2004;111:895–900 © 2004 by the American Academy of Ophthalmology.*

Stevens–Johnson syndrome (SJS) is a severe ocular surface disease with poor visual prognosis.[1–3] Symblepharon, adhesive occlusion of the lacrimal puncta, and corneal opacification with conjunctivalization are often observed in the chronic stages of the disease.[4] Severe dry eye due to the absence of reflex tearing is another major problem in such patients,[5] leading to worsening of ocular surface health.[2,6]

Tear supplementation with artificial eyedrops and autologous serum drops is important for the management of ocular surface diseases because autologous serum provides wetting and essential tear components, such as epidermal growth factor (EGF)[7] and vitamin A.[6,8,9] In addition to tear supplementation, some patients benefit from suppression of the ocular surface inflammation by topical corticosteroids and cyclosporin eyedrops.[10,11] Interestingly, we have also observed that those SJS patients with punctal occlusion due to symblepharon or scarring benefited from the complication and had healthier ocular surface epithelia. We thus evaluated the efficacy of lacrimal punctal occlusion, which we thought to be useful in retaining the tears and essential tear components, thereby promoting the normal maturation and proliferation of the ocular surface epithelium.

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Materials and Methods

Patients

Thirty-one eyes of 18 patients (9 male and 9 female; mean age: 39.6 ± 17.5 years [range: 12–71]) with SJS seen at the Dry Eye Subspecialty Outpatient Clinic of the Department of Ophthalmology at Tokyo Dental College were identified for this study. The diagnosis of SJS was based on a history of the presence of cryptogenic fever and acute inflammation of mucosal membranes after taking antibiotic or anti-inflammatory drugs, and on the presence of the chronic phase of an ocular complication such as symblepharon, entropion, trichiasis, xerophthalmia, and/or peripheral corneal vascularization.[2,6,12] Patients with diseases other than SJS that would affect the ocular surface adversely, such as atopic dermatitis, atopic or vernal allergic keratoconjunctivitis, and ocular–cicatricial pemphigoid; drug toxicity; chemical, thermal, or radiation injury; or any other ocular or systemic disorder that would create an ocular surface problem were excluded from this study. Before the lacrimal punctal occlusion procedure, all patients used topical medications, including preservative-free artificial tears, preservative-free hyaluronic acid, 0.1% betamethasone or 1% methylprednisolone eyedrops, and/or 0.05% cyclosporin. The frequency of eyedrops was adjusted according to the individual needs of the patients. Autologous serum drops (20%) were applied in 15 eyes. Therapeutic contact lenses were used in 4 eyes.

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Lacrimal Punctal Occlusion Procedure

When the ocular surface medical management was judged insufficient for alleviating patients' symptoms and objective ocular surface epithelial damage, punctal occlusion was added. Informed consent related to the method and effect of punctal occlusion and possible complications was obtained from all participants. An ethical board review and permission were obtained for the punctal occlusion procedure and the tests.

Initial topical medication was not changed during the treatment period with punctal occlusion, to allow for better assessment of the validity of the treatment.

We occluded both the upper and lower lacrimal puncta by cauterization or with silicone punctum plugs.[13] Six eyes with occlusion of the upper or lower lacrimal puncta from complications of SJS received lacrimal punctal occlusion in the open punctum only.

Lacrimal punctum cauterization was performed in 30 eyes. We injected 2% lidocaine hydrochloride in the skin and the conjunctiva surrounding the punctum. We then inserted a disposable battery-operated ophthalmic cautery tip (OPTEMP, Alcon Laboratories, Fort Worth, TX) into the punctum and along the distal portion of the canaliculus, and cauterized until the tissue around the punctum shrank and turned white, which took approximately 5 to 10 seconds. Oxytetracycline eye ointment was applied postoperatively.

We also studied the rate of recanalization in our cases. In eyes with punctal recanalization, we performed recauterization. Postoperative ocular surface data were also obtained from patients 3 months after a successful punctal occlusion procedure.

The changes in objective findings with punctal occlusion were compared before and 3 months after the treatment. Patients were asked about the changes in their dry eye symptomatology, such as foreign body sensation, burning, and their feeling of wetness of their eyes after punctal occlusion. The improvement of the subjective ocular symptoms after the treatment period was graded as worse, no change, or improved. Eight eyes with no improvement in the subjective and objective clinical findings within 4 weeks of punctal occlusion received autologous serum eyedrops or therapeutic contact lenses in addition to their regular regimen. The data from these eyes were evaluated before punctal occlusion, at the time of the institution of autologous serum eyedrops and therapeutic contact lenses, and at the final visit. Best-corrected Landolt visual acuities (VAs) were measured before and after lacrimal punctal occlusion as well.

Tear Function and Ocular Surface Evaluation

The standard Schirmer test without topical anesthesia was performed. The standardized strips of filter paper (Alcon Laboratories) were placed in the lateral canthus away from the cornea and left in place for 5 minutes with the eyes closed. Readings were reported in millimeters of wetting for 5 minutes.

The tear clearance test was performed for 5 minutes after placing a 10-μl drop of fluorescein sodium 0.5% combined with oxybuprocaine chloride 0.4% in the conjunctival sac of each eye. Subjects were asked to open their eyes for 5 minutes. Standard Schirmer strips were inserted with both eyes closed for another 5 minutes. The intensity of the staining was compared with a standard color plate for the Schirmer test. The tear clearance rate was determined by the rate at which color of the 0.5% fluorescein faded, and graded as 1×, 2×, 4×, 8×, 16×, 32×, 64×, 128×, or 256×.[14] Any value greater than 8× was considered normal. The ocular surface was assessed by the double vital staining method.[15] A 2-μl volume of a preservative-free combination of 1% Rose Bengal and 1% fluorescein dye was placed in the conjunctival sac. The Rose Bengal staining scores of the ocular surface ranged between 0 and 9 points.[16] Any score above 3 points was regarded as abnormal. The fluorescein staining was recorded in the upper, middle, and lower areas of the cornea, with a maximum score of 9 points.[8]

Statistical Analysis

A paired t test was used for the analysis of the Schirmer test, tear clearance rates, and staining scores before and after punctal occlusion. A probability level of $P<0.05$ was considered statistically significant.

Results

Clinical Findings

The clinical features of the patients are summarized in Table 1. Six eyes underwent prior ocular surface reconstruction, including excision of abnormal tissue surfacing the cornea, limbal allograft, and amniotic membrane transplantation. Penetrating keratoplasty was performed in 2 cases. Nineteen (61.3%) of 31 eyes were reported to have symptomatic improvement. None of the patients reported worsening of the symptoms. Patients who had symptomatic improvement could also decrease the frequency of artificial tear eyedrops. None of the patients developed epiphora after the surgery. Five eyes with no improvement in the subjective and objective clinical findings within 4 weeks of punctal occlusion received autologous serum eyedrops, and 3 other eyes received therapeutic contact lenses.

The average of logarithm of the minimum angle of resolution VA significantly improved from −0.64 to −0.52 ($P<0.05$). Figure 1 shows the changes of VA before and after the treatment period.

Tear Function Parameters

The Schirmer I test results before punctal occlusion yielded a mean score of 7.4±8.7 mm, which changed to 10.2±8.3 mm after punctal occlusion. The relation was not statistically significant ($P>0.05$). The mean preocclusion and postocclusion tear clearance values were 7.5±6.6 times and 4.9±4.8 times, respectively ($P<0.05$). The change of tear function parameters is summarized in Table 2.

Twenty-two eyes (71%) had improvement of Rose Bengal staining scores after punctal occlusion. The Rose Bengal staining score decreased from a mean of 4.7±2.8 points to 2.7±2.2 points ($P<0.05$). Twenty-three of 31 eyes (74.2%) showed an improvement of their fluorescein staining scores. The fluorescein staining score decreased from a mean of 5.0±2.3 points to 2.2±2.5 points ($P<0.05$). The Rose Bengal staining score of the 5 eyes that received additional autologous serum eyedrops after punctal occlusion decreased from a mean of 5.4±1.9 points to 2.6±2.7 points at the last follow-up, whereas the fluorescein staining score decreased from a mean of 4.8±4.6 points to 4.6±1.3 points. The Rose Bengal staining score of the 3 eyes that received additional disposable contact lenses decreased from a mean of 3.7±2.3 points to 1.3±2.3 points, and the fluorescein staining score decreased from a mean of 3.7±0.6 points to 2.0±3.5 points. The pre–punctal occlusion and post–punctal occlusion Rose Bengal staining scores of the 23 eyes that did not receive any additional autologous serum or contact lenses were 4.3±2.8 points and 2.0±1.8 points ($P<0.05$). Likewise, the preocclusion and postocclusion fluorescein staining scores for these 23 eyes were 4.6±2.2 points and 1.4±1.6 points ($P<0.05$). Figure 2 shows the anterior segment and

Table 1. Profile of the Patients with Stevens-Johnson Syndrome

Patient	Age (yrs)	Gender	Laterality	BCVA	Vascularization of Cornea	Synechial Punctal Occlusion	Prior Surgery	Schirmer I Test (mm)	Tear Clearance Test (×)	Additional Treatment
1	12	M	RE	20/25	Moderate	L	—	5	1	—
			LE	20/32	Moderate	—	—	6	2	—
2	43	F	RE	20/32	Moderate	L	—	8	1	Serum
3	48	M	RE	20/25	Moderate	—	—	1	2	Serum
			LE	20/25	Moderate	—	—	2	2	Serum
4	52	F	LE	20/32	Severe	—	—	0	16	Serum
5	56	M	LE	20/40	Severe	U	—	7	1	—
6	16	F	RE	20/25	Moderate	—	—	0	16	—
			LE	20/25	Severe	—	—	0	8	—
7	65	F	RE	20/20	Moderate	—	—	1	2	—
			LE	20/20	Moderate	—	—	2	1	—
8	56	M	RE	20/20	Moderate	—	—	5	4	Serum, CL
			LE	20/32	Severe	—	LT/AMT/Ts	6	1	—
9	30	M	LE	HM	Severe	—	—	0	2	CL
10	26	M	RE	20/100	Severe	—	—	2	16	CL
			LE	20/100	Severe	—	—	1	16	—
11	34	M	RE	20/125	Severe	—	—	5	2	—
			LE	20/630	Severe	—	—	28	2	—
12	42	M	RE	20/16	Moderate	—	—	13	4	Serum
			LE	20/63	Severe	—	LT	0	12	Serum
13	25	F	RE	20/20	Moderate	L	—	11	4	—
			LE	20/20	Moderate	L	—	5	4	—
14	40	M	RE	1/100	Severe	—	—	20	16	—
			LE	2/100	Severe	—	—	2	16	—
15	12	F	RE	20/16	Moderate	—	—	7	16	Serum
			LE	20/400	Severe	L	—	18	12	Serum
16	71	F	RE	20/400	Moderate	—	LT/AMT	35	16	Serum
			LE	20/320	Moderate	—	LT/AMT/PK	20	16	Serum
17	33	F	RE	20/12	Moderate	—	—	12	16	Serum
			LE	20/320	Severe	—	Resection of tissue	2	1	Serum
18	52	F	RE	20/320	Severe	—	LT/PK/PEA/PCIOL	5	4	Serum, CL

AMT = amniotic membrane transplantation; BCVA = best-corrected visual acuity; CL = contact lens; HM = hand movements; L = lower; LE = left eye; LT = limbal transplantation; moderate = peripherally vascularized corneas; PCIOL = implantation of posterior chamber intraocular lens; PEA = phacoemulsification and aspiration; PK = penetrating keratoplasty; RE = right eye; serum = autologous serum eye drops; severe = midperipheral or central vascularization; Ts = tarsorrhaphy; U = upper.

fluorescein staining patterns of a representative case of SJS before and after punctal occlusion.

Complications

The complications related to lacrimal punctal cauterization were severe pain in one patient and granuloma formation in another. The granuloma was seen to respond to topical corticosteroid treatment within 3 months. Lacrimal punctal recanalization was observed in 6 (26.1%) of the 23 eyes that had received punctal occlusion only, without any additional intervention. The change of Rose Bengal and fluorescein staining scores for eyes with punctal recanalization are shown in Tables 3 and 4.

Discussion

Lacrimal punctal occlusion has been reported to be a simple, safe, and effective procedure to treat aqueous tear deficiency and ocular surface epitheliopathies associated with penetrating keratoplasty, superior limbic keratoconjunctivitis, neurotrophic keratopathy, recurrent corneal erosions, and toxic epitheliopathy.[13,17] Although ocular surface epitheliopathy is also encountered in SJS, there are only a few reports about the management of the ocular surface disease in SJS. We performed lacrimal punctal occlusion in patients with SJS in whom we judged the medical management of the ocular surface disorder to be insufficient for the alleviation of patients' symptoms and objective ocular surface damage. We found that ocular surface Rose Bengal and fluorescein vital staining scores improved significantly with lacrimal punctal occlusion. Symptomatic improvement was seen in 61.3% of the eyes, and no eyes exhibited worsening of the symptoms. We believe that lacrimal punctal occlusion provided retention of available tears and lubrication, thus preventing mechanical ocular surface microtrauma and desiccation from blinking. Lacrimal punctal occlusion may have also helped by maximizing the time that essential tear components are in contact with the ocular surface epithelium. Substances present in tears, such as vitamin A, EGF, and transforming growth factor α, function to maintain a normal corneal and conjunctival epithelium. Vitamin A is known to suppress keratinization and promote normal differentiation of corneal epithelial cells. Epidermal growth factor and transforming growth factor α are thought to be

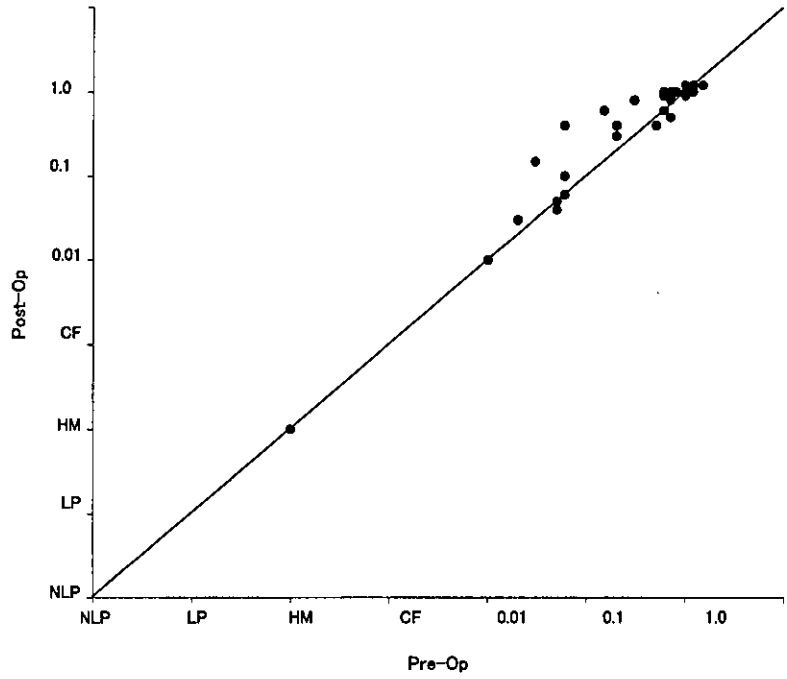

Figure 1. Change of best-corrected visual acuity (VA) with punctal occlusion. The diagonal line indicates the values at which the preoperative (Pre-Op) and postoperative (Post-Op) values for VA were the same. Visual acuity after punctal occlusion was improved in 15 eyes, and the same vision was maintained in 5 eyes. Its average was significantly improved. 1.0 = 20/20; 0.1 = 20/200; 0.01 = 1/100; CF = counting fingers; HM = hand movements; LP = light perception; NLP = no light perception.

important in controlling the turnover of the corneal epithelium and wound healing. Thus, a better supply of vitamin A, EGF, and transforming growth factor α by punctal occlusion might have helped in attaining better ocular surface vital staining scores.

Stevens–Johnson syndrome is an intractable disease that, we believe, must be managed with all possible efforts, including other treatment modalities such as autologous serum use and/or therapeutic contact lens applications. Indeed, we used autologous serum eyedrops and/or contact lenses in only those patients with an inadequate response to lacrimal punctal occlusion, which helped in the recovery of ocular surface health. Autologous serum contains vitamin A, EGF, acidic and basic fibroblastic growth factors, fibronectin, serum antiproteases such as α_2-macroglobulin, and neural factors, which promote epithelial healing and migration.[18–20] Autologous serum has been reported to provide these essential healers in higher amounts and also to increase mucin expression by the ocular surface and improve the vital staining scores.[8]

Table 2. Tear Function Changes with Punctal Occlusion

	Before Punctal Occlusion	After Punctal Occlusion
Schirmer I test (mm)	7.4 ± 8.7	10.2 ± 8.3
Tear clearance rate (×)	7.5 ± 6.6*	4.9 ± 4.8
Rose Bengal score	4.7 ± 2.8*	2.7 ± 2.2
Fluorescein score	5.0 ± 2.3*	2.2 ± 2.5

*$P<0.05$, paired t test.

In this study, we noted with surprise that lacrimal punctal occlusion was associated not only with improvement of ocular surface health parameters but with improvement of the best-corrected VA in 50% of the patients as well. We thought that lacrimal punctal occlusion provided an optically better corneal surface, and that the visual improvement may have resulted from increased regularity of the ocular surface due to epithelial healing and increased tear film stability, as evidenced by improvement of vital staining scores. Indeed, Goto et al reported that the topographical surface regularity index is elevated in severe dry eye patients, including SJS subjects, and that these patients all have decreased functional VA.[21] An investigation into the changes of topographical regularity indices in SJS patients with punctal occlusion would be very interesting.

We did not observe important complications with lacrimal punctal occlusion other than pain with insertion in one patient and granuloma in another. However, 26.1% of the patients showed recanalization at 3 months. Long-term studies assessing the relationship of this complication to ocular surface health parameters are definitely essential. We also remind the readership of this article that clinicians should be very careful about toxic epitheliopathy associated with punctal occlusion due to accumulation of preservatives on the ocular surface. Therefore, preservative-free medications should be chosen after such procedures. Long-term and larger prospective studies comparing the differential ocular surface effects of lacrimal punctal cauterization and

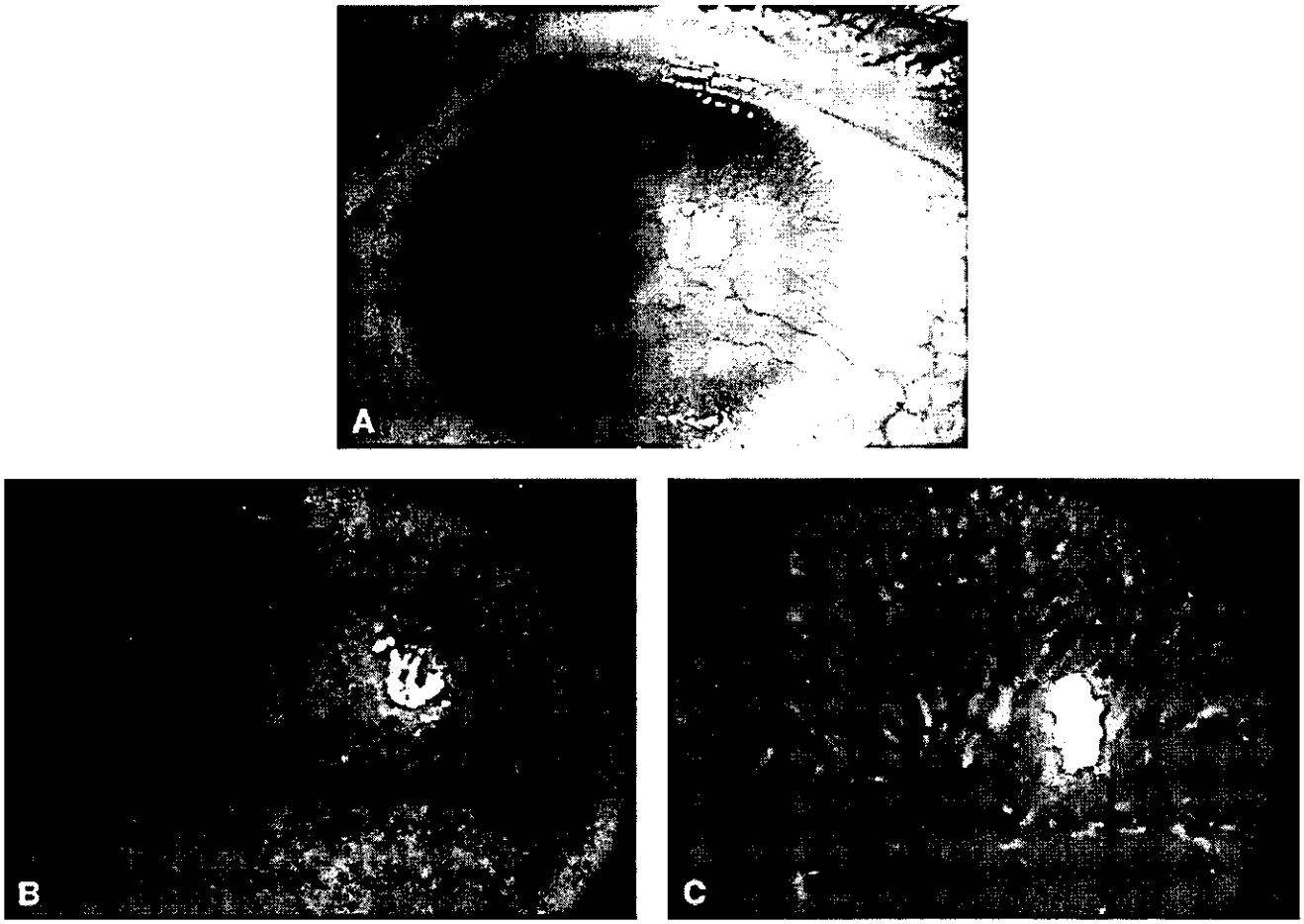

Figure 2. Photographic change of ocular surface with punctal occlusion. **A,** The anterior segment photograph of a 26-year-old patient with Stevens–Johnson syndrome. **B,** Corneal fluorescein staining of the same patient before punctal occlusion. **C,** Fluorescein staining pattern after punctal occlusion. Note that the fluorescein staining has almost disappeared, although the corneal surface is still irregular.

plug occlusion on corneal sensitivity, vital staining scores, tear meniscus, and cytology parameters are also needed. It would be interesting to see studies on the additive effects of different agents such as topical retinoids, mucin solutions, and the eicosanoid 15-S-HETE on the ocular surface of patients with SJS after lacrimal punctal occlusion (Cornea 19[suppl 2]:122, 2000).[22,23] In conclusion, we found lacrimal punctal occlusion to be of benefit for the management of ocular surface disease in chronic SJS. We tried to provide some understanding of the tear function changes in such patients, and believe that more studies should be carried out along the indicated lines.

Table 3. Change of Vital Staining Scores in Eyes with Punctal Recanalization (n = 6 Eyes)

	Before Recanalization	After Recanalization
Rose Bengal score	2.0±1.9	4.0±2.0
Fluorescein score	1.7±1.5	3.2±2.5

Table 4. Change of Vital Staining Scores in Eyes without Punctal Recanalization (n = 17 Eyes)

	Before Punctal Occlusion	After Punctal Occlusion
Rose Bengal score	3.8±2.2*	1.9±2.0
Fluorescein score	5.0±2.2*	1.4±1.7

*$P<0.05$, paired t test.

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A New Noninvasive Tear Stability Analysis System for the Assessment of Dry Eyes

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Purpose. This was a prospective case-control study conducted to evaluate the effectiveness of the Tear Stability Analysis System (TSAS) for the assessment of patients with dry eye.

Methods. The TSAS can take 10 consecutive corneal topograms at one per second for 10 seconds. Examinations using the TSAS were conducted in 26 eyes of 26 healthy control subjects and in 27 eyes of 27 patients with dry eye. Examinations were also conducted in 14 eyes of 14 patients before and after the insertion of punctum plugs. Surface regularity and asymmetry indices (SRI, SAI), as well as new tear stability regularity and asymmetry indices (TSRI, TSAI), derived from SRI and SAI, were analyzed.

Results. The mean SRI and SAI in dry eyes were significantly greater than in control eyes ($P < 0.05$). The time-wise change of SRI and SAI was significantly different between dry eyes and control eyes ($P < 0.05$). TSRI and TSAI in dry eyes were also significantly greater than in control eyes. Punctum plug insertion was associated with a significant decrease in SRI and SAI ($P < 0.05$).

Conclusions. TSAS was effective in objectively assessing the tear stability in patients with dry eye. This system may be useful in noninvasive diagnosis of dry eye and evaluation of treatment effects. (*Invest Ophthalmol Vis Sci.* 2004;45: 1369-1374) DOI:10.1167/iovs.03-0712

A stable and continuous tear film is essential for achieving an optically smooth surface. Prolonged gaze without blinking results in tear film instability, which has been reported to be associated with decreased functional visual acuity and worsening of the surface regularity index (SRI) in conventional corneal topography in patients with dry eye.[1]

The stability of tear film depends on many factors, such as intact blink reflex mechanisms, presence of healthy lacrimal glandular tissue, and structurally intact tear film. All three layers of the tear film—namely, the mucin, aqueous, and lipid layers—significantly contribute to tear stability. Qualitative and quantitative disorders of any of these layers can affect the tear film stability immensely.

Tear film stability can be analyzed by invasive techniques that use fluorescein, such as tear film break-up time (BUT) and tear clearance tests,[2] as well as noninvasive methods including tear film lipid layer interferometry,[3,4] tear evaporation test, and BUT assessment by using a grid xeroscope.[5]

BUT analysis with fluorescein dye is the most commonly used test of tear stability. Although it is easy to perform, variations in the concentration and pH of the fluorescein solution, the volume of the instilled drops, the presence of preservatives and invasiveness of the procedure are the main sources of error in this method. Research into noninvasive methods resulted in the development of techniques for the assessment of precorneal tear film BUT without the use of fluorescein (noninvasive BUT). Noninvasive BUT using instruments such as the grid xeroscope or tearscope (Keeler, Windsor, UK) allowed evaluation of the tear film by eliminating physical disturbance of the film from the instillation of fluorescein, along with the possibility of reflex tearing. Noninvasive BUT has been reported to be valuable for the diagnosis of tear film mucous layer deficiencies. However, noninvasive BUT did not find widespread acceptance in clinical practice due to problems in quantification of tear film stability.

Corneal topographical examinations have also been shown to help in the assessment of corneal surface irregularities in aqueous tear deficiency states, by using indices such as SRI and SAI.[6,7] These indices represent local variations in corneal contour providing invaluable information about the relation of the corneal and tear film status with progression of corneal disease and irregular astigmatism. However, conventional corneal topography examination methods can provide corneal surface data at only one time point. We developed a software program and called it the Tear Stability Analysis System (TSAS) for the TMS-2N corneal topography instrument (Tomey Technology, Nagoya, Japan), which can take 10 consecutive corneal topograms, one per second for 10 seconds. TSAS can detect subtle time-wise changes in the tear film deriving data from the distortion of the mire rings. Goto et al.[8] recently reported that TSAS could detect subtle tear film instability in eyes with normal BUT, by using fluorescein dye.

In this study, we performed TSAS measurements in patients with dry eye, to evaluate the effectiveness of this new system in the diagnosis of tear film instability, and compared the results with those in normal control subjects. We also assessed the changes in tear stability in subjects with dry eye who were treated with punctum plugs, to investigate the applicability of TSAS in the evaluation of dry eye management with punctal occlusion.

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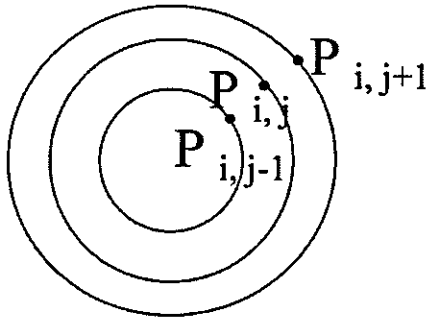


$$SRI = \ln\left\{ A * \frac{\sum_{i=1,256}^{j=2,10} \left[P_{i,j} - \left(P_{i,j+1} + P_{i,j-1} \right) / 2 \right]}{N} \right\} - B$$

FIGURE 1. The definition of SRI. *A*, *B*, scaling constants; $P_{i,j}$, the matrix of corneal power; *i*, the hemimeridian index; *j*, the mire ring number; *N*, total number of powers within the ±1.0-D window.

METHODS

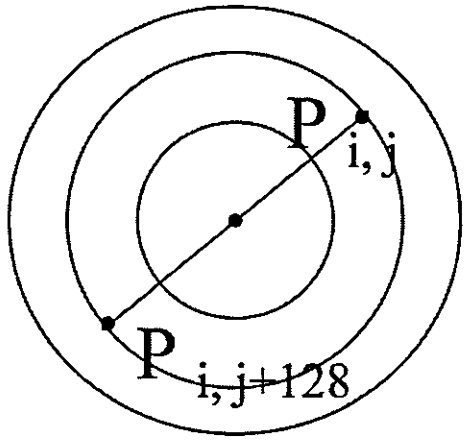
Monocular TSAS examinations were performed on 17 right eyes of 17 patients with non-Sjögren syndrome (non-SS; 5 men and 12 women; average age, 57.8 ± 8.4 years) and 10 eyes of 10 patients with Sjögren's syndrome (SS; 3 men and 7 women; average age, 62.1 ± 7.2 years). Twenty-six right eyes of 26 healthy control subjects also underwent the same examinations (8 men and 18 women; average age, 56.4 ± 9.4 years). There were no statistically significant age and sex differences between the control subjects and patients in this study. The research adhered to the tenets of the Declaration of Helsinki, and informed consent was obtained from all the subjects after explanation of the nature and possible consequences of the study.

Tear Function Diagnostic Criteria and Ocular Surface Evaluations

The diagnosis of dry eye was based on the diagnostic criteria of the Dry Eye Research Group in Japan.[9,10] In brief, patients with dry eye-related symptoms, positive staining with fluorescein or rose bengal, and Schirmer 1 test results of less than 5 mm or tear BUT of less than 5 seconds were diagnosed to have dry eye. The ocular surface was examined by the double vital staining method.[11] Two microliters of preservative-free combination of 1% rose bengal and 1% fluorescein dye was instilled in the conjunctival sac. Rose bengal staining of the ocular surface was scored according to the criteria proposed by van Bijsterveld[12] and fluorescein staining was scored according to the protocol described by Shimmura et al.[13] A fluorescein staining score above 1 point and a rose bengal staining score of more than 3 points was considered abnormal. BUT was measured three times, and the mean value was calculated.[11] Dry eye cases were categorized as non-SS and SS on the basis of the criteria proposed by Fox et al.[14] We recruited healthy control subjects with normal tear function and no vital staining of the ocular surface. The patients and control subjects did not have any history of ocular surgery, including punctal occlusion, ocular or systemic disease, or a history of drug or contact lens use that would alter the ocular surface. TSAS measurements were conducted before the tear function and vital staining examinations to minimize their effects on the TSAS testings. According to the study protocol, tear film BUT analysis was performed afterward, followed by fluorescein and rose bengal vital staining of the ocular surface. Schirmer I test was performed finally 1 hour after the initial TSAS testing to minimize the effect of the application of local anesthetic.

TSAS Measurements

We developed a new software called TSAS in collaboration with Department of Ophthalmology, Ehime University and Tomey Technology (Nagoya, Japan). TSAS was installed in the Topographic Modeling System (TMS-2N; Computed Anatomy, Tomey Technology). In TSAS testings, 30 mire rings are projected on the corneal surface. The projected images are captured by videokeratoscopy. The ring data are decomposed into 256 individual point data and analyzed by computer. Briefly, the center of the innermost mire is established as a reference point during testing. Having established a central reference point, coordinates are given to each data point where a semimeridian intersects a mire. The 256 equally spaced semimeridians theoretically provide approximately 6,000 to 11,000 data points. The actual number of data points available for analysis maybe reduced by mire distortion induced by shadows, position of the eyelids, corneal surface, and tear film irregularities. The coordinates locating the data points are converted to polar coordinates on the keratoscopy mires to facilitate corneal topographical reconstruction. A reconstruction algorithm is then applied to the location of each point on the two dimensional reflection. The algorithms reflect the shape of the cornea and are presented as statistical indices in topographic displays. Statistical indices are numbers that summarize a particular feature of the cornea. Among them, the surface regularity index (SRI) is the measure of the local regularity of the corneal surface within the central 4.5-mm diameter.[7] Within this area, the power of each point is compared with that of the points immediately surrounding it. This index has been reported to correlate well with visual function. The construction of the SRI algorithm is shown in Figure 1. In contrast, the surface asymmetry



$$SAI = \frac{\sum_{i=1}^{30} \left\{ \sum_{j=1}^{128} \left(P_{i,j} - P_{i,j+128} \right) * F_{ci} \right\}}{30 * \sum_{i=1}^{30} F_{ci}}$$

FIGURE 2. The definition of SAI. *Symbols* are as in Figure 1, with the addition of F_{ci}, which is a scaling constant corresponding to the mire ring.

TABLE 1. Tear Function and Vital Staining Scores

	Schirmer (mm)	BUT (sec)	FL	RB
Dry eye and control				
Dry eye (n = 27)	7.5 ± 4.6	2.6 ± 1.2	3.8 ± 2.7	4.6 ± 2.9
Control (n = 26)	14.3 ± 3.8	12.8 ± 3.4	0.0 ± 0.0	0.0 ± 0.0
Non-SS and SS				
Non-SS (n = 17)	10.5 ± 5.9	2.5 ± 1.3	2.9 ± 2.3	3.7 ± 2.6
SS (n = 10)	4.8 ± 3.1	1.7 ± 0.8	5.0 ± 2.1	5.8 ± 3.1
Before and after punctum plug				
Before plug (n = 14)	5.7 ± 2.7	2.3 ± 1.2	4.4 ± 2.7	5.3 ± 2.8
After plug (n = 14)	8.3 ± 3.3	3.6 ± 1.5	1.8 ± 2.3	2.6 ± 1.2

Data provided as (mean ± SD).

FL, score by fluorescein dye; RB, score by rose bengal dye; non-SS, non-Sjögren syndrome; SS, Sjögren syndrome; Before plug, before punctal plug insertion; After plug, after punctal plug insertion.

index (SAI) is the measure of the difference in corneal powers between points on the same ring 180° apart.[15] The power distribution across a normal corneal surface is fairly symmetric, and this index has been reported to increase in corneal disorders indicating progression of the disease state. Figure 2 shows construction of the SAI algorithm in detail. During TSAS measurements, corneal topograms were taken every second for 10 seconds after opening the eyes. To be able to perform the TSAS measurement without blinking, we applied 30 μL of 0.4% oxybuprocaine to the eyes to anesthetize the ocular surface. After 5 minutes, patients and normal control subjects were asked to stop blinking and keep their eyes open while their tear stability was being measured. The same experienced examiner performed all TSAS measurements obtaining well-focused and properly aligned images of the eyes. Eleven consecutive images were displayed on one printout. Mean surface regularity (SRI) and surface asymmetry index (SAI) information was provided in the upper left and right areas of each image. The printouts also provided information on the time-wise transition of SRI and SAI. In this study, we defined two new tear stability indices, which were calculated as follows: (1) TSRI = maximum SRI − minimum SRI within 10 seconds; (2) TSAI = maximum SAI − minimum SAI within 10 seconds.

Kinetic measurements of ocular surface regularity after 10 seconds of sustained eye opening were performed in patients with dry eye and in normal control subjects. SRI, SAI, TSRI, and TSAI were compared between these groups. Likewise, the same comparisons were performed between the SS and non-SS groups, including comparisons before and after plug insertion.

Punctum Plug Procedure

Punctum plugs were inserted in 14 eyes of 14 patients with dry eye. Two types of punctum plugs (FCI plug; FCI Ophthalmics, Issy-Les-Moulineaux, France, and Eagleplug; Eagle Vision, Memphis, TN) were used in this study. Plugs were inserted in both superior and inferior lacrimal puncta. Tear function and ocular surface as well as TSAS measurements were performed before and 2 weeks after plug insertion.

Statistical Analyses

A two-way repeated-measures ANOVA was performed for the comparison of tear film and corneal surface regularity, as well as asymmetry index differences between the dry eye and control groups. The same test was instituted to test the index differences before and after the punctum plug treatment. The two- way repeated- measures ANOVA was used to assess the time-wise differences of surface irregularity and asymmetry indices with TSAS measurements. Student's *t*-test was used

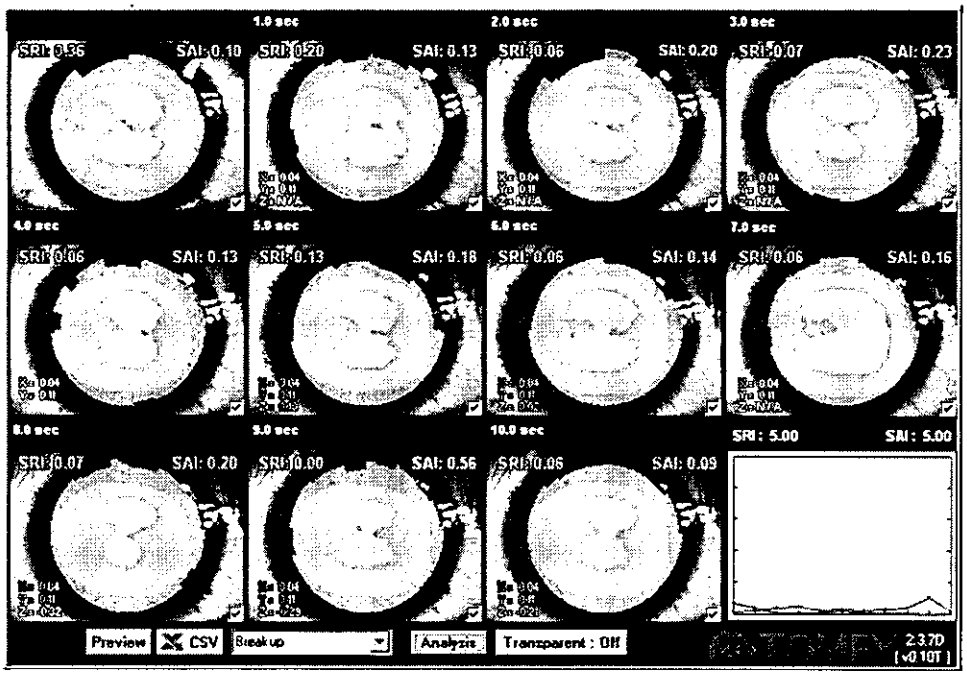


FIGURE 3. Representative TSAS map from a normal subject, a 38-year-old man who had a Schirmer test result of 10 mm. BUT was 12 seconds, and there was no vital staining score. Note the stability of the map as well as the SRI and SAI. *Inset*: SRI (*blue*) and SAI (*red*) transition with the eye open.

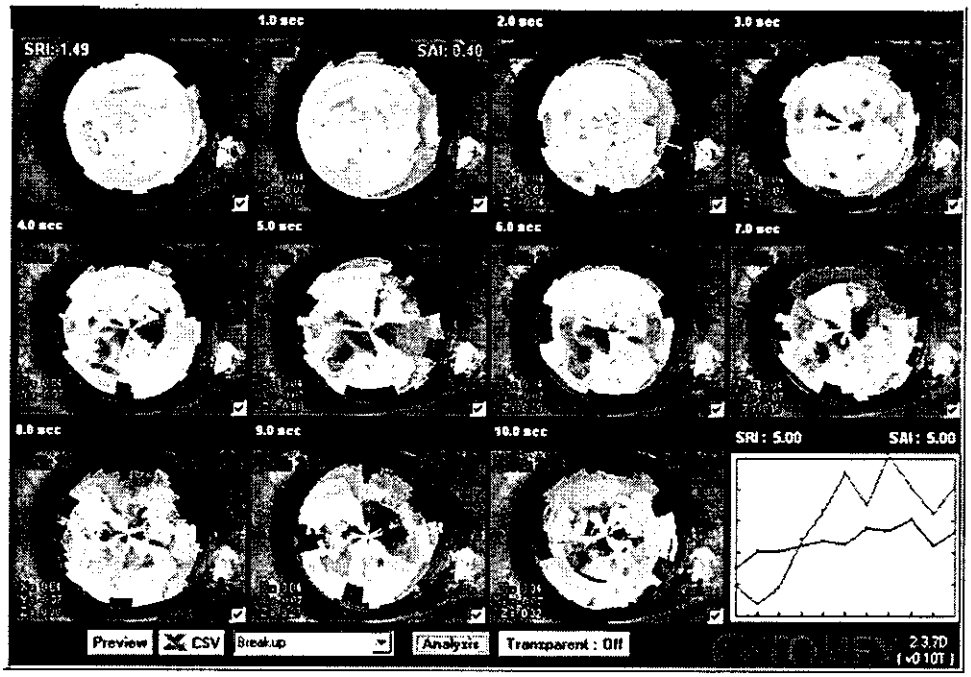


FIGURE 4. A representative TSAS map of a patient with dry eye, a 57-year-old man who had a Schirmer test result of 2 mm and BUT of 3 seconds. Rose bengal and fluorescein scores were both 6. Note the dramatic change in the TSAS pattern with time. *Inset*: the SRI (*blue*) and SAI (*red*) increased with time with the eye open.

for the comparison of TSRI and TSAI. A χ^2 test was applied to assess the age and sex differences between the control subjects and patients. $P < 0.05$ was considered statistically significant. A computer was used for statistical analysis (StatView software Windows 98/2000; SAS Institute Inc., Cary, NC).

RESULTS

Tear Function and Tear Stability Assessment of Patients with Dry Eye and Normal Subjects

The tear functions and vital staining scores of the subjects with dry eye and the control subjects are shown in Table 1. TSAS findings in a representative control subject with no considerable changes in the regularity and asymmetry indices are shown in Figure 3. Figure 4 demonstrates the gradual increase of the same indices in a patient with SS who had severe dry eye. TSAS examination showed that mean SRI and SAI from 0 to 10 seconds were significantly higher in the patients with dry eye than in the normal subjects ($P < 0.05$; Figs. 5, 6). The time-wise variations of SRI and SAI were also statistically significant within each group ($P < 0.05$). The TSRI and TSAI in the patients with dry eye were also significantly higher than in the normal control subjects (Table 2; $P < 0.05$).

Comparison of Tear Functions and Tear Stability between the SS and Non-SS Groups

The tear functions and vital staining scores of the SS and non-SS dry eye groups are shown in Table 1. TSAS examination showed that mean SRI and SAI from 0 to 10 seconds were significantly higher in patients with SS than in patients with non-SS dry eye ($P < 0.05$; Figs. 7, 8). The time-wise variation of the SRI from 0 to 6 seconds was observed to be significantly different between the SS and non-SS groups (Fig. 7; $P < 0.05$). Likewise, the time-wise variation of the SAI from 0 to 10

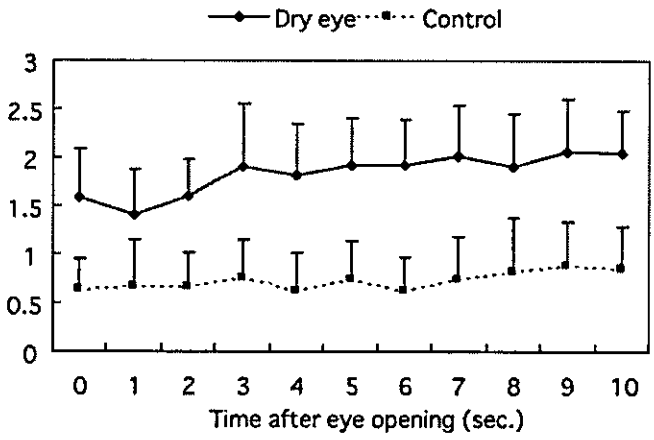


FIGURE 5. Comparison of SRI between patients with dry eye and control subjects. Error bars: standard deviation. The mean SRI from 0 to 10 seconds was significantly higher in the patients with dry eye than in the normal subjects ($P < 0.05$). The time-wise variations of SRI were also statistically significant within each group ($P < 0.05$).

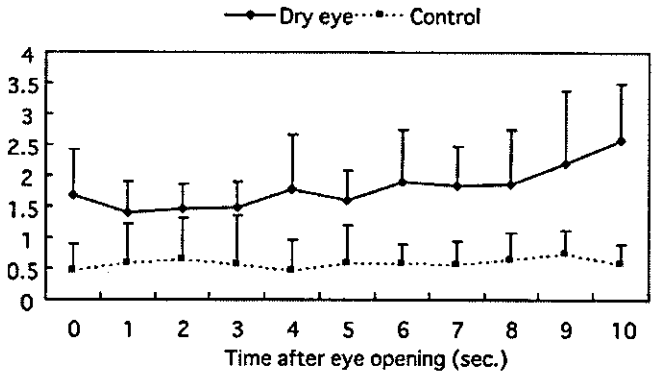


FIGURE 6. Comparison of SAI between patients with dry eye and control subjects. Error bars: standard deviation. The mean SAI from 0 to 10 seconds was significantly higher in the patients with dry eye than in the normal subjects ($P < 0.05$). The time-wise variations of SAI were also statistically significant within each group ($P < 0.05$).

TABLE 2. TSI of Patients with Dry Eye and Normal Control Subjects

Indices	TSRI	TSAI
Dry eye (n = 27)	1.3 ± 0.4*	2.1 ± 1.3*
Normal control (n = 26)	0.72 ± 0.3	1.1 ± 0.9

Data provided as (mean ± SD). TSRI, Tear Stability Regularity Index; TSAI, Tear Stability Asymmetry Index.
*$P < 0.05$.

seconds were observed to be significantly different between the SS and non-SS groups (Fig. 8; $P < 0.05$).

Comparison of Tear Function and Tear Stability before and after Punctum Plug Insertion

We observed no complications related to the punctum plug insertion. The tear functions and vital staining scores of the patients with dry eye before and after plug insertion are shown in Table 1. After punctum plug insertion, mean SRI and SAI from 0 to 10 seconds showed a significant improvement compared with the pretreatment indices ($P < 0.05$; Figs. 9, 10). The time-wise variations of SRI and SAI were not significantly different ($P > 0.05$).

DISCUSSION

The efficacy of the TSAS, using videokeratography as a noninvasive and an objective method of tear stability assessment, has already been established.[8] The only available study on tear film stability employing this new device analyzed tear film stability using parameters such as BUT and break-up area.[8] In this study, we used the TSAS to measure the kinetic tear stability changes in patients with dry eye and normal subjects by using SRI, SAI, and two new indices, TSAI and TSRI; analyzed the tear stability index differences between patients with SS and those with non-SS dry eye; and looked into the changes in stability indices with punctum plug treatment in patients with dry eye. We have reported that functional visual acuity in patients with dry eye significantly decrease with concomitant increase in SRI.[1] Decreased functional visual acuity in that study suggests impaired visual function on prolonged gaze in subjects with dry eye.

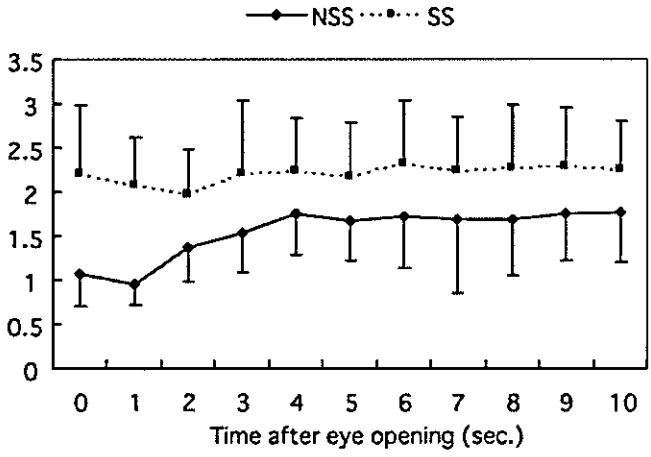


FIGURE 7. Comparison of SRI between the SS and non-SS groups. Error bars: standard deviation. The mean SRI from 0 to 10 seconds was significantly higher in the SS than in the non-SS group ($P < 0.05$). The time-wise variation of the SRI from 0 to 6 seconds was significantly different between the SS and non-SS groups as shown ($P < 0.05$).

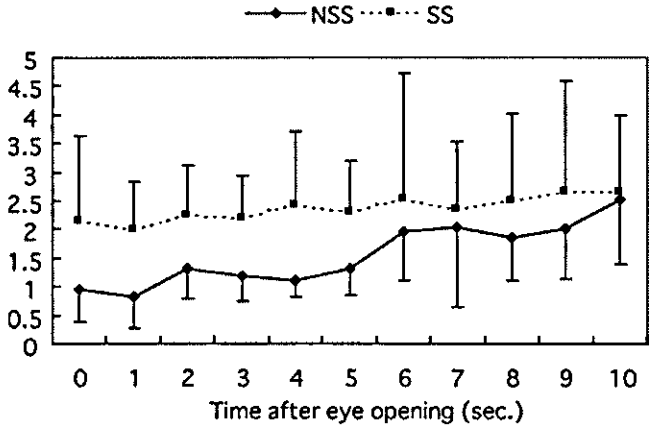


FIGURE 8. Comparison of SAI between SS and non-SS groups. Error bars: standard deviation. The mean SAI from 0 to 10 seconds was significantly higher in the SS than in the non-SS group ($P < 0.05$). The time-wise variation of the SAI from 0 to 10 seconds was significantly different between the SS and non-SS groups ($P < 0.05$).

The new TSAS allowed evaluation of the kinetic tear stability and corneal topographical changes during 10 seconds of blink-free gaze. TSAS assesses the changes of topographical mires reflected from the tear film surface. Although it is still not clear whether the tear stability changes measured by TSAS are due to complete full-thickness tear film disruption or discontinuity of the tear lipid layer, further studies in which TSAS and lipid layer interferometry are performed simultaneously on the same subset of patients with dry eye and control subjects will clarify these issues. TSAS examinations showed that the kinetic tear stability as assessed by SRI and SAI was significantly worse in patients with dry eye. Likewise, TSRI and TSAI fared significantly worse in the subjects with dry eye. Our previous attempts to performed TSAS measurements without topical anesthesia resulted in reflex tearing in some patients with dry eye who could not tolerate long blink-free periods. Thus, we used topical anesthesia in this study. It has been confirmed that a single application of oxybuprocaine does not affect the visual acuity or tear film stability itself in patients with dry eye or normal control subjects.[16-18]

We were surprised to find that the kinetic tear stability patterns in patients with SS or non-SS dry eye were different

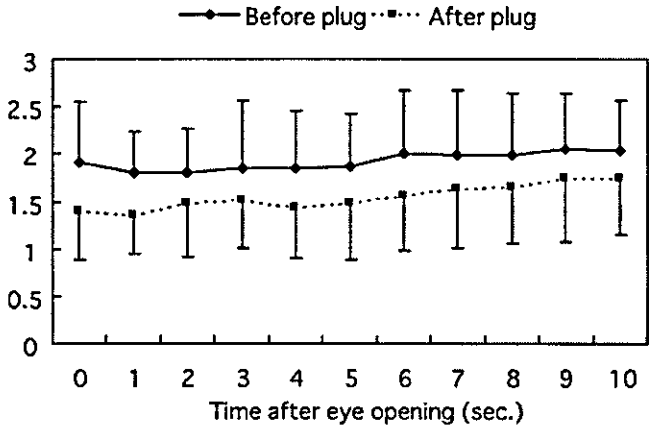


FIGURE 9. Comparison of SRI before and after punctum plug insertion. Error bars: standard deviation. The mean SRI from 0 to 10 seconds showed a significant improvement compared with the pretreatment indices ($P < 0.05$). The time-wise variations of SRI were not significantly different ($P > 0.05$).

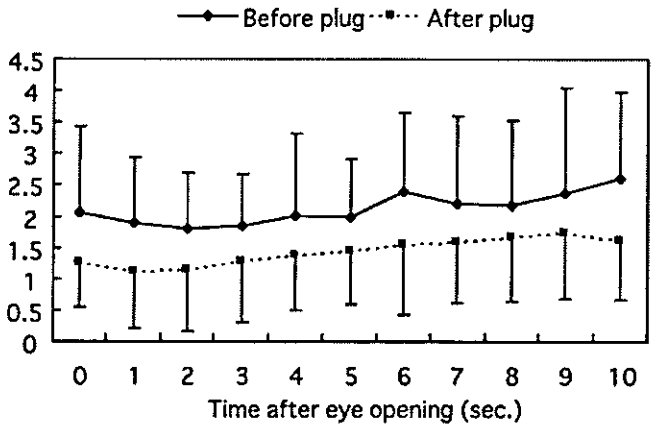


FIGURE 10. Comparison of SAI before and after punctum plug insertion. Error bars: standard deviation. Mean SAI from 0 to 10 seconds showed a significant improvement compared with the pretreatment indices ($P < 0.05$). The time-wise variations of SAI were not significantly different ($P > 0.05$).

from each other. The SRI and SAI indices were significantly lower initially in the non-SS subjects, in whom they increased within the first few seconds and displayed values closer to those in the patients with SS afterward. We believe that comparatively poorer tearing and a higher degree of corneal epithelial damage were responsible for the higher initial and consistently higher SRI and SAI scores in the patients with SS. Patients with non-SS dry eye had relatively better tearing and lesser vital staining scores with better kinetic stability, as evidenced by lower initial SRI and SAI scores, which worsened gradually after prolonged gaze without blinking. Although we did not quantify them in this study, we attributed the worsening of the surface regularity and asymmetry indices to the changes in tear evaporation. We observed that the magnitude of kinetic tear stability changes were much less in patients with SS compared with patients with non-SS dry eye. Thus, the new TSRI and TSAI were thought to be effective in the detection of the tear stability changes in patients with mild dry eye with low rose bengal and fluorescein staining scores. TSAS examination was also useful for the evaluation of dry eye management with punctum plugs. We expected a worsening of the pretreatment SRI and SAI in the patients with dry eye selected for punctal occlusion. However, the time-wise variation of the indices before occlusion did not show a marked change, probably because patients who underwent punctum plug treatment had considerably higher ocular surface vital staining scores than the overall group with dry eye in this study and because the SRI and SAI did not get worse during the testing. The SRI and SAI improved significantly with punctal occlusion, which suggests attainment of tear stability with treatment.

In conclusion, TSAS helped in the assessment of tear stability in patients with dry eye and seemed to be promising in differentiating types of dry eye by quantitatively evaluating the dynamic changes of tear stability over 10 seconds. TSAS was also useful in evaluating the effect of punctal occlusion therapy. We believe that studies with larger number of subjects that look into the correlation of tear clearance rate with the tear stability indices would be very interesting. Measurements of tear stability within shorter intervals will increase the sensitivity of noninvasive tear film stability evaluations by TSAS in the near future.

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Experimental Oral Medicine

Salivary flow and its relationship to oral signs and symptoms in patients with dry eyes

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OBJECTIVES: The aim of this study was to investigate oral symptoms and clinical parameters in dry eye patients. Subjective reports of the sensation of a dry mouth, salivary flow rates, and clinical parameters of oral disease related to three different types of dry eye patients were examined.

SUBJECTS AND METHODS: There were 224 individuals, including dry eye patients and control subjects. The dry eye patients were classified into three types: patients with Sjögren's syndrome (SS-DE), patients without SS-DE (non-SS-DE), and patients with Stevens–Johnson syndrome (SJS-DE). Salivary flow rates were measured using two kinds of sialometry. Subjective and objective oral symptoms and signs were also examined.

RESULTS AND CONCLUSION: Over half of the dry eye patients complained of a dry mouth. The flow rates of their stimulated whole saliva and parotid saliva were significantly lower than those of the control groups ($P < 0.05$, $P < 0.01$). The sensation of a dry mouth and changes in oral soft tissues, dental caries, and oral *Candida* frequently occurred in dry eye patients.

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Keywords: dry eye; dry mouth; salivary flow rate

Introduction

The prevalence of symptomatic associations between dry eye and dry mouth in population-based studies has already been reported (Hay *et al*, 1998; Schein *et al*, 1999; Price and Venables, 2002). Hay *et al* (1998) examined the prevalence of dry eye and dry mouth in 341 subjects and reported that 29% of subjects had dry mouth symptoms, 24% had dry eye symptoms and 14% had both. Hikichi *et al* (1995) reported that 17% of outpatients in general eye clinics have some signs or symptoms of dry eye. These patients often complain of dryness of mouth and condition of intra-oral changes.

Eye clinicians classify dry eye syndrome into three types: simple dry eye, autoimmune positive dry eye, and Sjögren's syndrome (SS) (Tsubota *et al*, 1994). Sjögren's syndrome is frequently associated with the occurrence of keratoconjunctivitis sicca and xerostomia as an autoimmune disorder (Fox *et al*, 1986). Stevens–Johnson syndrome (SJS) is also known to cause dry eye syndrome. Stevens and Johnson (1922) reported this to be a specific syndrome, which includes eruptive fever with stomatitis and ocular inflammation. Drug intake and infections can also affect the skin and mucous membranes and initiate these diseases. However, the mechanisms underlying such effects have not been identified (Coster, 1997).

As far as we know, no study has ever been undertaken to explore the relationship between dry eye and dry mouth with respect to the different types of dry eye patients. We therefore examined the relationship between the three types of dry eye patients and their oral signs and symptoms in dry eye patients.

Materials and methods

Study population

This study was conducted on dry eye outpatients at Ichikawa General Hospital, Tokyo Dental College in Chiba Prefecture, Japan. Written informed consent was obtained from each patient after the aims and methodology of the study were explained. The survey was carried out between June 1999 and August 2002. There were 141 dry eye patients, 17 men and 124 women, with a mean age of 56.01 ± 13.65 years (20–79). Dry eye patients were diagnosed based on the criteria used by the Japanese Dry Eye Research Center (Hikichi *et al*, 1995). We classified the dry eye patients into three groups: (1) dry eye patients with Sjögren's syndrome (SS-DE; $n = 54$) as diagnosed by Fox's criteria (Fox *et al*, 1986), Fox's criteria fit the recently revised EC-North

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American classification criteria (Vitali *et al*, 2002); (2) dry eye patients without Sjögren's syndrome (non-SS-DE; $n = 67$) (Lemp, 1995); and (3) dry eye patients with Stevens–Johnson syndrome, who were induced by medications (SJS-DE; $n = 20$). We also classified two control groups: (1) age-matched patients with ophthalmopathies such as cataract, myopia, keratoconus, and myodesopsia excluding dry eye symptoms [control patients (CP), $n = 32$]; (2) age-matched healthy volunteers receiving regular health checks in the Tokyo metropolitan area and in Chiba Prefecture [control (C), $n = 51$]. The mean ages of CP and C were 54.25 and 50.98, respectively (Table 1). The examinations of dry eye and dry mouth including the CP groups were carried out blind knowing that they have some ophthalmopathy. The oral health checks of healthy volunteers were also performed as blind to the examiner.

Saliva sampling
All the patients visited the dry eye clinics between 9:00 and 12:00 hours. Patients were forbidden any oral intake or smoking for at least 1 h prior to saliva collection. We examined the stimulated whole salivary secretion, followed by stimulated parotid saliva.

Sampling of stimulated whole saliva
Whole saliva was measured by stimulation using a chewing gum base (0.5 g) without sugar, flavour, or any additives. In order to soften the gum base and remove saliva from the mouth, the saliva secreted was swallowed after initial chewing for 1 min. During the subsequent 4 min, stimulated saliva was collected in a sterilized plastic tube while the subject chewed the same bolus gum base. The flow rate was recorded as $ml\ min^{-1}$. We excluded those patients who were wearing dentures. It is impossible to masticate the chewing gum base because of the adhesiveness of gum base to dentures. We excluded three patients of SS-DE, two of SJS-DE, seven of non-SS-DE, five of CP, and one-denture wearer in the control subjects.

Sampling of stimulated parotid saliva
Parotid saliva was stimulated using sour apricot candy (2.6 g; Lotte Co., Tokyo, Japan) and was collected for 5 min in a modified Lashley' cup (Lashley, 1916).

Table 1 Age distribution of subject groups

Subjects	*n*	*Mean ± s.d.*	*Range*
SS-DE[a]	54	58.09 ± 10.61	30–78
Non-SS-DE[b]	67	55.18 ± 14.50	20–79
SJS-DE[c]	20	52.85 ± 17.02	22–76
CP[d]	32	54.25 ± 13.23	25–78
C[e]	51	50.98 ± 15.03	30–78

[a]SS-DE, Sjögren's syndrome with dry eye; [b]non-SS-DE, dry eye not associated with Sjögren's syndrome; [c]SJS-DE, Stevens–Johnson syndrome with dry eye; [d]CP, control patients; [e]C, healthy volunteers as a control.

Oral examination
The examination was carried out by one of the authors (K.M.). Dental caries was registered with an explorer and mirror under standardized conditions and optimal light. Caries data were expressed as decayed, missing, and filled teeth (DMFT). The third molars were excluded from the analysis. To examine oral soft tissue (buccal mucosa, tongue mucosa and angular cheilitis), a calibration trial was conducted between the examiner and patients. Kappa statistic for the recording of oral soft tissue was more than 0.9. Buccal mucosa was examined as normal, slight redness, severe redness and leukoedema. Changes in the tongue mucosa were classified as normal, atrophy of tongue mucosa, groove of the tongue mucosa and shiny tongue. Angular cheilitis were classified as normal, slight angular cheilitis, heavy cheilitis or angular cheilitis at both corners of the mouth.

Detection of oral Candida
Oral mucosa (tongue, cheeks, pharynx), teeth and denture surfaces were swabbed using selective medium CA (Candida Color; Kanto Kagaku Co., Tokyo, Japan) (Okubo *et al*, 1997) to examine the distribution of *Candida* in the mouth. After incubation at 37°C for 48 h, the number of colonies was counted for four species (*Candida albicans*, *C. glabrata*, *C. tropicalis*, *C. krusei*) according to the manufacturer's instructions.

Questionnaire
The subjects were asked to answer a questionnaire consisting of questions relating to symptoms of their sensation of a dry mouth, their general medical condition, and usage of medication. Subjects were asked to indicate whether their symptoms were never, occasionally, or frequently experienced.

Statistical analysis
Data were statistically analysed using StatView (Version 5.0, SAS Institute, Cary, NC, USA). A two-tailed Fisher exact test and chi-square test were used to analyse the questionnaire. A one-way analysis of variance (one-way ANOVA) was used to evaluate the differences in the clinical parameters between the groups.

Results

Salivary flow rates in dry eye patients
In SS-DE, both stimulated whole and parotid saliva were the lowest volume in other subject groups and significant differences were observed between SS-DE *vs* CP and C in stimulated whole saliva ($P < 0.01$) and SS-DE *vs* non-SS-DE, CP, and C in stimulated parotid saliva ($P < 0.05$, $P < 0.01$). In non-SS-DE, there was a significant difference between CP and C in stimulated whole saliva ($P < 0.05$, $P < 0.01$). In SJS-DE, both stimulated whole and parotid saliva were lower compared with CP and C subjects. However, no significant difference was found between the groups (Table 2).