Results

A total of 138 eyes of 73 patients from the 3 institutions were included in this study. There were 33 males and 40 females. Their age ranged from 10 to 83 years (mean±standard deviation, 47.9±18.5 years). At disease onset, the patients' ages ranged from 2 to 69 years (mean±standard deviation, 28.4±18.2 years), and the duration of the illness before seeking consultation at our centers ranged from 1 to 54 years (mean±standard deviation, 18.8±15.5 years). Drugs were the most commonly associated etiologic factor in 47 patients (64.4%). Because 14 of these patients used 2 or 3 types of drugs simultaneously, it was difficult to identify the drug(s) implicated in disease onset; therefore, we considered all their drugs to be causative. The causative drugs were antibiotics in 21 patients, cold remedies in 18 patients, nonsteroidal antiinflammatory drugs in 10 patients, anticonvulsants in 6 patients, and other in 4 patients. The precise history regarding the use of drugs was unclear in 20 patients because of the long interval between disease onset and this study.

Corneal Complications

A detailed summary of the 7 evaluated components comprising corneal complications is shown in Table 1. Among the 138 eyes examined, 114 (82.6%) manifested a total loss of POV (grade 3). Moderate to severe (grade 2 or 3) corneal SPK was present in 93 eyes (67.4%), neovascularization was present in 83 eyes (60.1%), and conjunctivalization was present in 82 eyes (59.4%).

Conjunctival and Eyelid Complications

Among the 6 evaluated components that comprise conjunctival and eyelid complications, the meibomian glands were most frequently and most severely involved; 102 of the 138 eyes (73.9%) manifested grade 3 meibomian gland involvement (Table 2). The scores for punctal damage and mucocutaneous involvement also were high; grade 2 or 3 punctal damage was assigned to 93 eyes (67.4%), and grade 2 or 3 mucocutaneous involvement was assigned to 71 eyes (51.4%).

Eye Complications Independent of Ocular Surface Disorders

Cataract was observed in 11 of 138 eyes. Glaucoma was diagnosed in 4 eyes, none of which had central loss of visual fields. There were no other eye complications independent of ocular surface disorders.

Visual Acuity

The number of eyes in each of the 4 groups was fairly evenly distributed (Table 3). Of the 138 eyes examined, 74 (53.6%) had visual acuity worse than 20/200 (group 3, n = 32; group 4, n = 42). Only 28 eyes (20.3%) had visual acuity equal to 20/20 or better.

Table 1. Summary of Corneal Complications (138 Eyes)

Complication	Grade 0, no. (%)	Grade 1, no. (%)	Grade 2, no. (%)	Grade 3, no. (%)
Superficial punctate keratopathy	22 (15.9)	23 (16.7)	18 (13.0)	75 (54.3)
Epithelial defect	135 (97.8)	2 (1.4)	1 (0.7)	0 (0)
The loss of palisades of Vogt	21 (15.2)	3 (2.1)	0 (0)	114 (82.6)
Conjunctivalization	41 (29.7)	15 (10.9)	10 (7.2)	72 (52.2)
Neovascularization	35 (25.4)	20 (14.5)	22 (15.9)	61 (44.2)
Opacification	43 (31.2)	41 (29.7)	28 (20.3)	26 (18.8)
Keratinization	105 (76.1)	10 (7.2)	5 (3.6)	18 (13.0)

Table 2. Summary of Conjunctival and Eyelid Complications (138 Eyes)

Complications	Grade 0, no. (%)	Grade 1, no. (%)	Grade 2, no. (%)	Grade 3, no. (%)
Conjunctival complications				
Hyperemia	46 (33.3)	61 (44.2)	15 (10.9)	16 (11.6)
Symblepharon formation	40 (29.0)	54 (39.1)	21 (15.2)	23 (16.7)
Eyelid complications				
Trichiasis	42 (30.4)	41 (29.7)	44 (31.9)	11 (8.0)
Mucocutaneous junction involvement	16 (11.6)	51 (37.0)	34 (24.6)	37 (26.8)
Meibomian gland involvement	13 (9.4)	14 (10.1)	9 (6.5)	102 (73.9)
Punctal damage	36 (26.1)	9 (6.5)	15 (10.9)	78 (56.5)

Correlation between Visual Acuity and Grade of Complications

When we compared eyes with better (20/200 or better) and worse (worse than 20/200) visual acuity with respect to the scores obtained for each of the 13 components, we found that with the exception of epithelial defect, the scores differed significantly (Table 4).

We estimated the correlation coefficient between the visual acuity of the 138 eyes and the severity grade, scored from 0 to 3, of each of the 13 evaluated components in the 3 categories of complications. We found that all 13 components were correlated significantly with logMAR visual acuity; the correlation coefficient (R) ranged from 0.359 to 0.810 ($P<0.0001$); for corneal epithelial defects, the value was $R = 0.169$ ($P = 0.0473$; Table 5). Of all the scores, corneal neovascularization, opacification, and conjunctivalization were most highly correlated with poor vision ($R = 0.810$, $P<0.0001$; $R = 0.784$, $P<0.0001$; and $R = 0.726$, $P<0.0001$, respectively).

The statistical model for predicting logMAR visual acuity was calculated using a linear model with stepwise variable selection as follows: logMAR = −0.2573 + cataract × 0.4153 + POV × 0.2814 + SPK × 0.08551 + epithelial defect × 0.3018 + neovascularization × 0.3471 + opacification × 0.3202 + keratinization × 0.1347. This multivariable regression analysis showed that corneal neovascularization, opacification, keratinization, and cataract had a significant effect on logMAR visual acuity (Table 6). The predicted logMAR was correlated significantly with the actual logMAR visual acuity measured ($R = 0.960$, $P<0.0001$).

Overall Total Score

The mean overall total score for the 13 components was 19.3±9.5 (range, 0–35). As shown in Tables 3 and 4 and Figure 3, eyes with a higher total score had poorer vision. The averaged scores for the 4 visual acuity groups were: group 1, 5.86 (range, 0–19); group 2, 16.64 (range, 2–28); group 3, 23.31 (range, 15–33); and group 4, 27.45 (range, 18–35). Pearson's analysis clearly demonstrated that the total score was significantly correlated with logMAR visual acuity ($R = 0.806$, $P<0.0001$; Fig 3). The subtotal scores of 3 problem categories correlated with the overall total score (Fig 4).

Table 3. Ocular Complications and Visual Acuity of Stevens–Johnson Syndrome Patients

Complications	Visual Acuity			
	Group 1, 20/20 or Better, Average Grade	Group 2, 20/20 to 20/200, Average Grade	Group 3, 20/200 to 20/2000, Average Grade	Group 4, Worse than 20/2000, Average Grade
No. of eyes	28	36	32	42
Corneal complications				
SPK	0.82	1.92	2.40	2.78
Epithelial defect	0	0	0.03	0.07
Loss of POV	0.82	2.78	3.00	3.00
Conjunctivalization	0.11	1.36	2.59	2.76
Neovascularization	0.25	1.11	2.38	2.90
Opacification	0.11	0.61	1.66	2.31
Keratinization	0.04	0.11	0.50	1.26
Conjunctival complications				
Hyperemia	0.36	0.89	1.19	1.40
Symblepharon formation	0.18	0.97	1.19	2.07
Eyelid complications				
Trichiasis	0.57	1.08	1.38	1.50
Mucocutaneous junction involvement	0.79	1.56	1.91	2.10
Meibomian gland involvement	1.32	2.50	2.69	2.90
Punctal damage	0.50	1.78	2.65	2.58
Total score	5.86	16.64	23.31	27.45

POV = palisades of Vogt; SPK = superficial punctate keratopathy.

Discussion

Severe ocular surface disease arising from SJS or TEN is associated with significant visual morbidity.[1–4] The evaluation of ocular complications in these patients is extremely important, because ocular involvement often represents the only long-term complication of SJS. There is currently no established method for evaluating the spectrum of ocular manifestations arising from these diseases. In this study, we detailed the characteristic ocular complications in the chronic stage of SJS and developed a grading system to assess more objectively the extent and severity of 13 components of these ocular complications. To the best of our knowledge, this is the first study that specifically attempted to improve and standardize the evaluation of ocular complications in SJS.

As we set out to develop a grading system that could be used easily by ophthalmologists, we identified complications that were important and could be evaluated easily by simple slit-lamp examination. After several pilot studies, we eventually settled on 13 components of 3 categories of

Table 4. Comparison between Ocular Complications and Visual Acuity

Complications	Visual Acuity of 20/200 or Better, Average Grade	Visual Acuity Worse than 20/200, Average Grade	P Value
No. of eyes	64	74	
Corneal complications			
SPK	1.44	2.62	<0.0001
Epithelial defect	0	0.05	0.1208
Loss of POV	1.92	3.00	<0.0001
Conjunctivalization	0.81	2.69	<0.0001
Neovascularization	0.73	2.68	<0.0001
Opacification	0.39	2.03	<0.0001
Keratinization	0.08	0.93	<0.0001
Conjunctival complications			
Hyperemia	0.66	1.31	<0.0001
Symblepharon formation	0.63	1.69	<0.0001
Eyelid complications			
Trichiasis	0.86	1.45	0.0002
Mucocutaneous junction involvement	1.23	2.01	<0.0001
Meibomian gland involvement	2.02	2.81	<0.0001
Punctal damage	1.26	2.61	<0.0001
Total score	11.86	25.66	

POV = palisades of Vogt; SPK = superficial punctate keratopathy.

Table 5. Correlation Analyses between 13 Complications and Logarithm of the Minimum Angle of Resolution Visual Acuity

Complications	Coefficient	P Value
Neovascularization	0.810	<0.0001
Opacification	0.784	<0.0001
Conjunctivalization	0.726	<0.0001
Symblepharon formation	0.649	<0.0001
SPK	0.601	<0.0001
Loss of POV	0.550	<0.0001
Punctal damage	0.518	<0.0001
Mucocutaneous junction involvement	0.488	<0.0001
Keratinization	0.477	<0.0001
Meibomian gland involvement	0.453	<0.0001
Hyperemia	0.383	<0.0001
Trichiasis	0.359	<0.0001
Epithelial defect	0.169	0.0473

POV = palisades of Vogt; SPK = superficial punctate keratopathy.

Table 6. Multivariable Regression Analysis

Variables	Coefficient	95% Confidence Intervals	P Values
Intercept	−0.2573	−0.5449 to 0.0303	0.0786
Neovascularization	0.3471	0.2113–0.4849	<0.0001
Opacification	0.3203	0.1734–0.4672	<0.0001
Keratinization	0.1347	0.0281–0.2413	0.0142
Cataract	0.4153	0.0249–0.8057	0.0375
Loss of POV	0.2814	−0.0784 to 0.6412	0.1228
SPK	0.0855	−0.0296 to 0.2006	0.1423
Epithelial defect	0.3018	−0.1057 to 0.7093	0.1434

POV = palisades of Vogt; SPK = superficial punctate keratopathy.

complications that we considered important for the assessment of severe or cicatricial ocular surface disorders. We used a simple method for grading the severity of these complications; the components were assigned scores that reflected whether involvement was mild, moderate, or severe. This grading system was judged to be easy and convenient at the 3 participating ophthalmology centers that evaluated 138 eyes from 73 SJS patients. The results obtained at the 3 centers were consistent and comparable. Ours is one of few prospective studies on the ocular complications of SJS, and each patient was evaluated carefully by at least 2 ophthalmologists. To the best of our knowledge, this is the largest such study reported to date.

The initial ocular pathologic process in SJS, inflammation and necrosis of the conjunctiva, often is accompanied by the destruction of goblet cells.[4,13,14] The production of mucin by these cells is vital for maintaining an adequate tear film essential for corneal clarity. Dry eye secondary to goblet cell destruction is the most common long-term ocular complication in patients with various ocular surface diseases.[4,13,14] Cicatricial lid and conjunctival complications include symblepharon formation, fornicеal shortening, keratinization, lid malposition (e.g., entropion), and misdirected eyelashes (trichiasis).[4,13,15–22] Limbal stem cell destruction, evidenced by loss of the POV, also may occur at disease onset and may be accompanied by severe inflammation. The combination of these complications may result in recurrent corneal erosion, ulceration, vascularization, stromal scarring, conjunctivalization of the corneal surface, and progressive corneal melting and perforation.[4,13,15–22]

In our study, drugs were the most commonly identified etiologic factor; in 47 patients (64.4%), antibiotics (n = 21 patients), cold remedies (n = 18 patients), or nonsteroidal antiinflammatory drugs (n = 10) were the causative agents. These findings are consistent with previous reports.[15,16,19,23]

Of all the complications, severe (grade 3) meibomian gland involvement and loss of the POV (102 and 114 eyes, respectively) were the most common ocular complications of SJS. We found that the total score for each eye was correlated significantly with its visual acuity; consistently, eyes with higher overall scores had poorer vision. We categorized the complications as those involving predominantly the cornea, the conjunctiva, and the eyelid. As expected, corneal complications were most likely to have a detrimental effect on vision. In particular, corneal neovascularization and opacification were correlated highly with posttreatment visual acuity in the chronic stage. Conjunctivalization, a sequela of limbal stem cell deficiency, also was correlated with poor vision in our series.

In our study, there was a high rate of lid complications in chronic SJS. Of the eyelid complications, meibomian gland involvement was moderate or severe (grade 2 or 3) in 111 of the 138 eyes (80.4%). We found that eyes without apparent corneal complications also manifested cicatricial eyelid changes. As such, the meibomian glands seem to be susceptible to injury after SJS. Because the meibomian glands play a critical role in the stabilization of the tear film, this is likely to contribute to the disruption of the tear film and severe dry eye condition experienced in patients in the chronic stage of SJS.

The use of a standardized method for grading the extent and severity of ocular complications in SJS patients offers

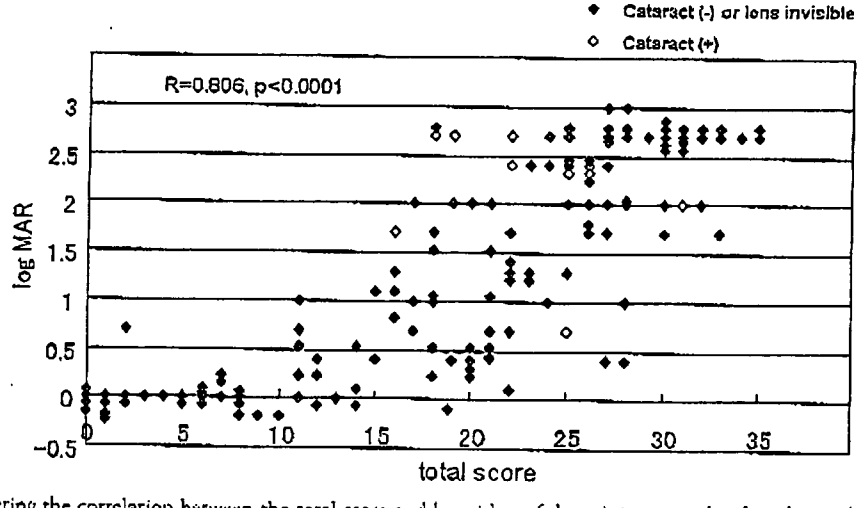

Figure 3. Scatterplot depicting the correlation between the total score and logarithm of the minimum angle of resolution (logMAR) visual acuity. The overall total score of 13 components (0–39) versus logMAR showed a significant positive correlation (Spearman $R = 0.806$, $P<0.0001$).

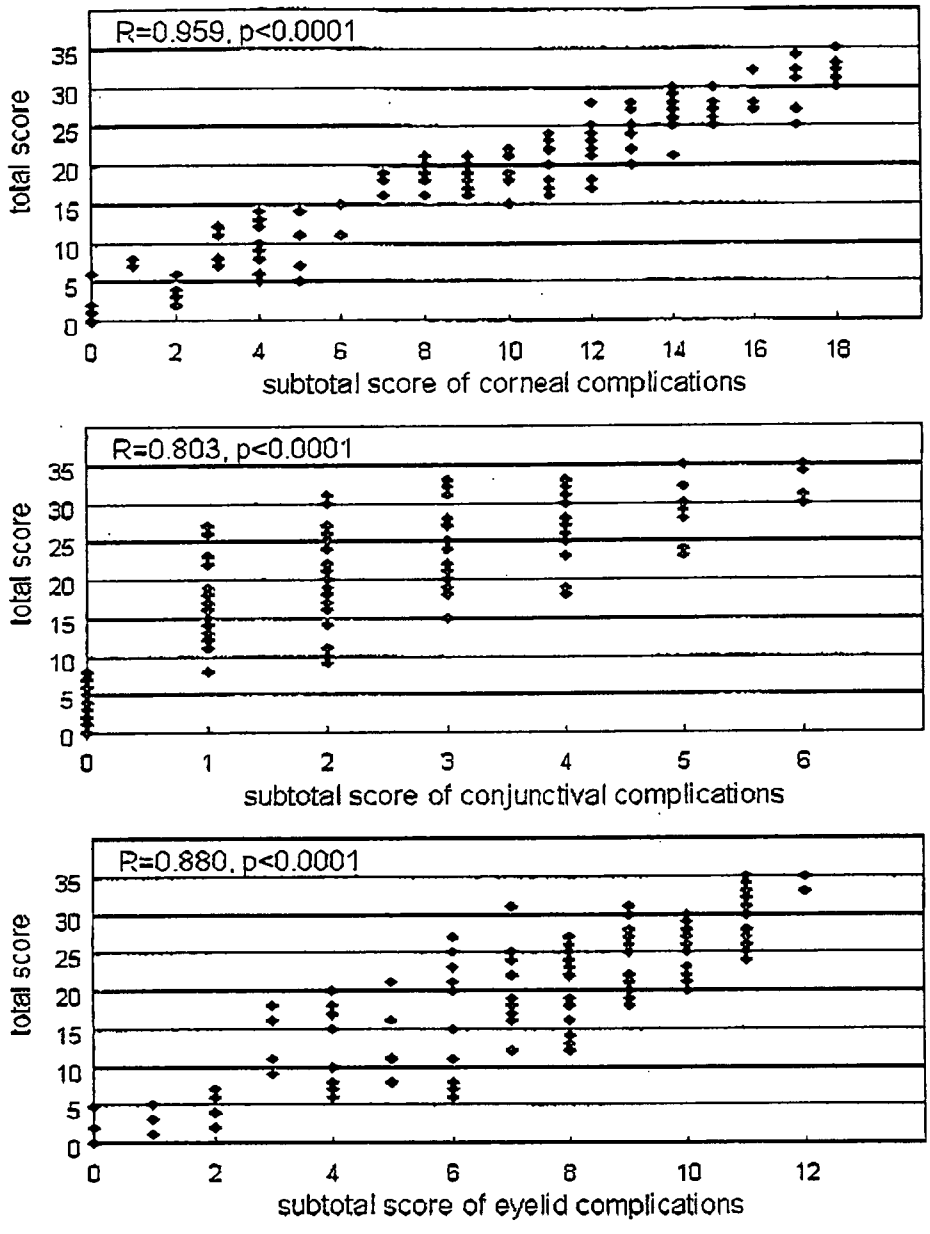

Figure 4. Scatterplots depicting the correlations between subtotal score of 3 categories and overall total score. Subtotal scores of corneal, conjunctival, and eyelid complications versus the total score all showed a significant positive correlation: (top) Spearman $R = 0.959$, $P<0.0001$; (middle) $R = 0.803$, $P<0.0001$; (bottom) $R = 0.880$, $P<0.0001$.

significant advantages. The grading system introduced here can be used in the initial evaluation and the follow-up and monitoring of ocular complications in SJS patients. As documented here, the lid margin is a commonly affected site in the disease process. However, because attention often focuses on the ocular surface, changes in the lid margin may be overlooked. Our grading system ensures that important ocular complications are detected by corneal specialists as well as nonspecialized ophthalmologists.

Ocular surface reconstructive procedures such as limbal and cultivated epithelial stem cell transplantation have been used to treat severe ocular manifestations in SJS patients.[8–11,24] However, because many of the reported studies are nonrandomized case series without control arms and because there is currently no standardized method for grading ocular complications in SJS patients in the acute and chronic stage, it is difficult to compare the treatment outcomes of these studies. Our grading system also provides a standardized method for evaluating patients before corneal and ocular surface transplantation procedures. The use of an objective method of grading the severity of the patient's preoperative condition ultimately may help in prognosticating the long-term clinical outcome of these eyes after surgery.

This is the first study that describes a method for classifying and grading the severity of ocular involvement in SJS patients. Our findings have important clinical implications and facilitate the objective evaluation of patients with ocular complications resulting from SJS. The method presented here may be adapted for use in patients with cicatricial ocular surface diseases arising from other causes such as ocular cicatricial pemphigoid and chemical injury. It also provides a common platform for the discussion and management of patients with ocular surface disorders and may be useful for predicting treatment outcomes. Our method also enables ophthalmologists to monitor more objectively the progression of complications during the follow-up of these patients.

References

1. Howard GM. The Stevens-Johnson syndrome: ocular prognosis and treatment. Am J Ophthalmol 1963;55:893–900.
2. Arstikaitis MJ. Ocular aftermath of Stevens-Johnson syndrome. Arch Ophthalmol 1973;90:376–9.
3. Wright P, Collin JR. The ocular complications of erythema multiforme (Stevens Johnson syndrome) and their management. Trans Ophthalmol Soc U K 1983;103(Pt 3):338–41.
4. Lehman SS. Long-term ocular complication of Stevens-Johnson syndrome. Clin Pediatr (Phila) 1999;38:425–7.
5. Auquier-Dunant A, Mockenhaupt M, Naldi L, et al. Correlations between clinical patterns and causes of erythema multiforme majus, Stevens-Johnson syndrome, and toxic epidermal necrolysis: results of an international prospective study. Arch Dermatol 2002;138:1019–24.
6. Roujeau JC. Stevens-Johnson syndrome and toxic epidermal necrolysis are severity variants of the same disease which differs from erythema multiforme. J Dermatol 1997;24: 726–9.
7. Assier H, Bastuji-Garin S, Revuz J, Roujeau JC. Erythema multiforme with mucous membrane involvement and Stevens-Johnson syndrome are clinically different disorders with distinct causes. Arch Dermatol 1995;131:539–43.
8. Kenyon KR, Tseng SC. Limbal autograft transplantation for ocular surface disorders. Ophthalmology 1989;96:709–22; discussion 722–3.
9. Thoft RA. Keratoepithelioplasty. Am J Ophthalmol 1984;97: 1–6.
10. Tseng SC, Prabhasawat P, Barton K, et al. Amniotic membrane transplantation with or without limbal allografts for corneal surface reconstruction in patients with limbal stem cell deficiency. Arch Ophthalmol 1998;116:431–41.
11. Koizumi N, Inatomi T, Suzuki T, et al. Cultivated corneal epithelial stem cell transplantation in ocular surface disorders. Ophthalmology 2001;108:1569–74.
12. Miyata K, Amano S, Sawa M, Nishida T. A novel grading method for superficial punctate keratopathy magnitude and its correlation with corneal epithelial permeability. Arch Ophthalmol 2003;121:1537–9.
13. Nelson JD, Wright JC. Conjunctival goblet cell densities in ocular surface disease. Arch Ophthalmol 1984;102:1049–51.
14. Ohji M, Ohmi G, Kiritoshi A, Kinoshita S. Goblet cell density in thermal and chemical injuries. Arch Ophthalmol 1987;105: 1686–8.
15. Rogers M, Rogers PA. Ocular involvement in the Stevens-Johnson syndrome. Australas J Dermatol 1981;22:89–90.
16. Wilkins J, Morrison L, White CR Jr. Oculocutaneous manifestations of the erythema multiforme/Stevens-Johnson syndrome/toxic epidermal necrolysis spectrum. Dermatol Clin 1992;10:571–82.
17. Bastuji-Garin S, Rzany B, Stern RS, et al. Clinical classification of cases of toxic epidermal necrolysis, Stevens-Johnson syndrome, and erythema multiforme. Arch Dermatol 1993; 129:92–6.
18. Cote B, Wechsler J, Bastuji-Garin S, et al. Clinicopathologic correlation in erythema multiforme and Stevens-Johnson syndrome. Arch Dermatol 1995;131:1268–72.
19. Power WJ, Saidman SL, Zhang DS, et al. HLA typing in patients with ocular manifestations of Stevens-Johnson syndrome. Ophthalmology 1996;103:1406–9.
20. Rzany B, Hering O, Mockenhaupt M, et al. Histopathological and epidemiological characteristics of patients with erythema exudativum multiforme major, Stevens-Johnson syndrome and toxic epidermal necrolysis. Br J Dermatol 1996;135:6–11.
21. Paquet P, Pierard GE. Erythema multiforme and toxic epidermal necrolysis: a comparative study. Am J Dermatopathol 1997;19:127–32.
22. Leaute-Labreze C, Lamireau T, Chawki D, et al. Diagnosis, classification, and management of erythema multiforme and Stevens-Johnson syndrome. Arch Dis Child 2000;83:347–52.
23. Roujeau JC, Kelly JP, Naldi L, et al. Medication use and the risk of Stevens-Johnson syndrome or toxic epidermal necrolysis. N Engl J Med 1995;333:1600–7.
24. Tsubota K, Satake Y, Ohyama M, et al. Surgical reconstruction of the ocular surface in advanced ocular cicatricial pemphigoid and Stevens-Johnson syndrome. Am J Ophthalmol 1996;122:38–52.

The Disease Burden of Keratoconus in Patients' Lives: Comparisons to a Japanese Normative Sample

Yukako Tatematsu-Ogawa, M.D., Masakazu Yamada, M.D., Motoko Kawashima, M.D., Yoshihiko Yamazaki, Ph.D., Thomas Bryce, M.D., and Kazuo Tsubota, M.D.

Eye & Contact Lens 34(1): 13–16, 2008

The Disease Burden of Keratoconus in Patients' Lives: Comparisons to a Japanese Normative Sample

Yukako Tatematsu-Ogawa, M.D., Masakazu Yamada, M.D., Motoko Kawashima, M.D., Yoshihiko Yamazaki, Ph.D., Thomas Bryce, M.D., and Kazuo Tsubota, M.D.

Purpose. Keratoconus is a chronic, noninflammatory, degenerative disease of the cornea that has an onset in young adulthood. The objective of this study was to evaluate vision-related quality of life (VR-QOL) in patients with keratoconus by using the Japanese version of the National Eye Institute Visual Function Questionnaire-25 (NEI-VFQ-25). **Methods.** Forty-five patients diagnosed with keratoconus at the Keio University School of Medicine were enrolled. Patients were divided into three subgroups according to corrected visual acuity. Group A included patients whose best-corrected visual acuity was at least 20/20 in both eyes. Group B included patients with a best-corrected visual acuity of at least 20/20 in only one eye. Group C included patients whose best-corrected visual acuity was worse than 20/20 in both eyes. Thirty-six age-matched subjects were recruited as control subjects. The Japanese version of the NEI-VFQ-25 was administered to each subject. **Results.** All NEI-VFQ-25 subscale scores were significantly lower ($P<0.05$) in patients with keratoconus than in the control subjects. Subscales evaluating general health, ocular pain, and vision-specific mental health showed particularly low values. Among patients with keratoconus, every subscale score other than color vision correlated with corrected visual acuity. **Conclusions.** The results support that and describe how multidimensional visual function and VR-QOL are impaired in patients with keratoconus, including those with normal visual acuity. Ophthalmologists and other clinicians should carefully evaluate and address the full range of quality of life issues that may affect patients with keratoconus.

Key Words: Contact lens—Keratoconus—Keratoplasty—Quality of life.

Keratoconus is a chronic, noninflammatory, degenerative disease of the cornea that has an onset in young adulthood and is characterized by a cone-shaped bulging of the corneal surface and stromal thinning.[1] Although some patients with keratoconus require keratoplasty to restore visual acuity, most patients are able to maintain normal visual acuity with the aid of contact lenses, although some patients experience contact lens intolerance.

The clinical status of patients with ocular disorders such as keratoconus is often evaluated through tests of objective function, such as visual acuity, refraction, and keratometric analysis. The past few decades have seen an increasing appreciation of the importance of assessing the illness experience in patients with ocular and other diseases in a comprehensive fashion that extends beyond measures of biologic status. Consequently, the concept of health-related quality of life (QOL) has been developed, which encompasses not only the biologic status of disease, but also the full range of complex effects that the illness and its various sequelae have on the life of the patient. Such sequelae may include the practical effects of the disease on the daily life of the patient, the side effects of treatment, issues of identity as the patient reconceives of himself or herself as an individual with a chronic illness, and social challenges involved in managing the disease and meeting the reactions of others to the illness. In this vein, in ophthalmology, concepts such as quality of vision and vision-related quality of life (VR-QOL) have been recently developed and are increasingly used. Several questionnaires have been developed to measure VR-QOL.[2–5]

Vision-related quality-of-life instruments can be divided into two broad categories: generic instruments, which are designed to be used in a broad spectrum of ocular disorders, and disease-specific instruments, which target patients with specific conditions. The National Eye Institute Visual Function Questionnaire-25 (NEI-VFQ-25), a generic instrument, has been intensively investigated, and its validity has been established for various ocular disorders, including glaucoma, age-related macular degeneration, diabetic retinopathy, cytomegalovirus retinitis, and age-related cataracts.[6–13] The NEI-VFQ-25 evaluates not only visual function and limitations in daily activities related to impaired visual function, but also the impact of ocular disease on patients' lives from various standpoints.[6] The NEI-VFQ-25 has been translated into several languages, including Japanese. The reliability and the validity of the Japanese version of the NEI-VFQ-25 are considered comparable to those of the English version.[14,15]

From the Department of Ophthalmology (Y.T-O., M.K., K.T.), Keio University School of Medicine, Tokyo, Japan; the Division for Vision Research (Y.T-O., M.Y.), National Institute of Sensory Organs, National Tokyo Medical Center, Tokyo, Japan; and the Department of Health Sociology (Y.Y., T.B.), Tokyo University Graduate School of Medicine, Tokyo, Japan.

The authors have no proprietary interest in any materials in this article.

Address correspondence and reprint requests to Dr. M. Yamada, Division for Vision Research, National Institute of Sensory Organs, National Tokyo Medical Center, 2-5-1 Higashigaoka, Meguro, Tokyo 152-8982, Japan; e-mail: yamadamasakazu@kankakuki.go.jp

Accepted February 23, 2007.

DOI: 10.1097/ICL.0b013e3180515282

Visual acuity is the most common measure of clinical status in patients with keratoconus. It is apparent, however, that normal visual acuity alone cannot ensure good QOV and VR-QOL in patients with keratoconus. In addition to possibly diminished visual acuity, patients with keratoconus also experience the anxiety of knowing that they may someday need to undergo surgery, such as keratoplasty, and of knowing that their condition may progress and possibly lead to low vision. They also endure the burden of frequent hospital visits. Additionally, most patients who depend on contact lenses experience anxiety because of decreased vision when they are not able to wear their lenses. Many patients with keratoconus have less than all-day wear with contact lenses because of irritation and have significantly reduced vision with glasses. Thus, it may be appropriate to use a more comprehensive QOL-related tool in the examination of these patients.[16]

This study sought to evaluate self-reported VR-QOL in patients with keratoconus by using the NEI-VFQ-25. The influence of disease severity on VR-QOL was also examined.

MATERIALS AND METHODS

Subjects

A total of 45 Japanese patients with bilateral keratoconus (12 women and 33 men) were enrolled. The average age was 36.3 ± 9.4 years (range, 20–63 years). All patients had been diagnosed with keratoconus at Keio University Hospital. Keratoconus diagnoses were made based on slitlamp examination findings and videokeratography. The patient sample included contact lens wearers, spectacle wearers, and patients with a history of keratoplasty in one eye. Patients with a history of other ocular diseases, such as retinal disease, cataracts, or glaucoma, were excluded from the study. Patients with a history of keratoplasty complications, such as allograft rejection, infection, and graft failure, were also excluded.

Patients were divided into three subgroups according to corrected visual acuity. Group A included patients whose best-corrected visual acuity in each eye was at least 20/20. Group B included patients with a best-corrected visual acuity of less than 20/20 in one eye. Group C included patients whose best-corrected visual acuity was less than 20/20 in both eyes. Thirty-six age-matched healthy Japanese subjects with no known ocular disorders other than refractive error were recruited as control subjects.

The principles of the Declaration of Helsinki were followed. Each subject received a thorough explanation of the purpose of the study and all procedures involved in the study and provided written informed consent before enrollment. Approval for this investigation was granted by the Committee for the Protection of Human Subjects at the Keio University School of Medicine.

The NEI-VFQ-25

The NEI-VFQ-25 consists of 25 core and 13 optional items. It is divided into the following 12 subscales: general health, general vision, ocular pain, near vision, distance vision, vision-specific social functioning, vision-specific mental health, vision-specific role difficulties, vision-specific dependency, driving, color vision, and peripheral vision. A self-administered Japanese version of the NEI-VFQ-25 was used in this study. The instrument generally took approximately 10 minutes for patients to complete and was administered to patients before the ophthalmologic examination.

TABLE 1. *Characteristics of Patients With Keratoconus and Control Subjects*

Characteristic	Patients with keratoconus (n = 45)	Control subjects (n = 36)
Mean age (years)	36.3 ± 9.4	36.3 ± 13.0
Gender		
Male	33 (73%)	23 (64%)
Female	12 (27%)	13 (36%)
Best-corrected visual acuity		
≥20/20 in both eyes	12 (27%)	36 (100%)
≥20/20 in one eye	26 (58%)	0
<20/20 in both eyes	7 (15%)	0
Means of refractive correction		
None	1 (2%)	10 (28%)
Spectacles	2 (4%)	10 (28%)
Soft contact lenses	1 (2%)	13 (36%)
Hard contact lenses	41 (92%)	3 (8%)
History of keratoplasty	12 (27%)	0

In the NEI-VFQ-25, each of its subscales is scored from 0 to 100, with higher scores representing better function. Clinical data, including patient age, sex, ocular and medical history, and history of ocular surgery, were obtained from medical records and patient interviews.

Statistical Analysis

Descriptive statistics were used and included measurement of means and dispersion. Descriptive data are presented as mean ± standard deviation and percentages. The Mann–Whitney test was used to compare the NEI-VFQ-25 scale scores between groups. To minimize bias, subscale scores were included in analyses only if all questions within the subscale were completed. All statistical analyses were performed with the SAS/STAT module of the SAS statistical software package (SAS Institute, Inc., Cary, NC).

RESULTS

Baseline demographic characteristics of patients with keratoconus and control subjects, including any means of refractive correction used, are shown in Table 1. Most (91%) patients with keratoconus were hard contact lens wearers. There were 12 patients in group A, 26 patients in group B, and seven patients in group C.

The NEI-VFQ-25 subscale scores of patients with keratoconus and control subjects are shown in Table 2. All subscale scores of patients with keratoconus were significantly lower than those of control subjects (P<0.01, Mann–Whitney test). Particularly low subscale scores were seen for patients with keratoconus in the general health, general vision, ocular pain, and vision-specific mental health subscale categories.

The NEI-VFQ-25 subscale scores of groups A, B, and C and control subjects are shown in Figure 1. Increasing visual acuity, as assessed by group A through C category membership, correlated with increasing QOL or function scores in all subscales among patients with keratoconus. Even in group A, in which the best-corrected visual acuity was 20/20 or better in both eyes, all subscale scores other than general health and driving were significantly lower than those of age-matched control subjects (P<0.05, Mann–Whitney test).

TABLE 2. *VFQ-25 Subscale Scores of Patients With Keratoconus and Control Subjects*

Subscale	Patients with keratoconus (n = 45)	Control subjects (n = 36)
General health	56.3 ± 18.6[a]	71.2 ± 17.6
General vision	62.3 ± 16.9[a]	81.4 ± 11.7
Ocular pain	57.4 ± 23.8[a]	83.0 ± 13.6
Near vision	80.5 ± 15.8[a]	92.8 ± 9.7
Distance vision	73.7 ± 16.1[a]	89.0 ± 13.3
Vision-specific		
Social functioning	85.9 ± 13.7[a]	97.2 ± 7.04
Mental health	65.9 ± 23.8[a]	90.9 ± 13.8
Role difficulties	77.8 ± 19.6[a]	96.3 ± 9.3
Dependency	88.2 ± 11.9[a]	98.3 ± 5.3
Driving	72.3 ± 20.6[a]	79.1 ± 24.2
Color vision	92.4 ± 11.4[a]	98.6 ± 5.7
Peripheral vision	74.6 ± 23.3[a]	92.4 ± 13.1

[a]$P<0.01$ for keratoconus–control comparisons by the Mann–Whitney test.

DISCUSSION

All mean NEI-VFQ-25 subscale scores for patients with keratoconus were significantly lower than those of age-matched control subjects ($P<0.01$, Mann–Whitney test), which was in good accordance with the report of Kymes et al.[16] Additionally, subscale scores correlated with corrected visual acuity as assessed by group A through C category membership. The finding that visual function subscale scores, such as general vision, near vision, distance vision, and driving are decreased in patients with keratoconus is intuitive. Most patients in this study had a corrected visual acuity of less than 20/20 in at least one eye, and many patients additionally may have subtle visual disturbances not evident on standard acuity testing. The low QOL scores observed on the ocular pain subscale may be explained by the need for many patients with keratoconus to wear hard contact lenses. Finally, the notably low mental health subscale scores found among patients with keratoconus warrants attention, suggesting that the various burdens of living with keratoconus may pose a considerable challenge to patient mental health and that practitioners should be attuned to this possible issue when treating keratoconus. These factors may explain the personality trends in keratoconus.[17,18] Through attention to such larger implications of disease, the care of patients with keratoconus may be guided toward helping them gain control of and most productively adapt to the illness experience.

All visual function subscale scores in group A, other than general health and driving, were significantly decreased compared with control subjects ($P<0.05$, Mann–Whitney test). Such patients notably showed low QOL scores also in the general health and mental health subscales. This supports the hypothesis that apparently normal visual acuity does not necessarily reflect unimpaired VR-QOL in patients with keratoconus.

The results underscore the complex nature of vision- and ocular-related QOL in patients with keratoconus. Patients with keratoconus experience multidimensional visual function impairment and associated QOL issues, and parameters other than traditional measures of visual acuity appear necessary to evaluate VR-QOL in

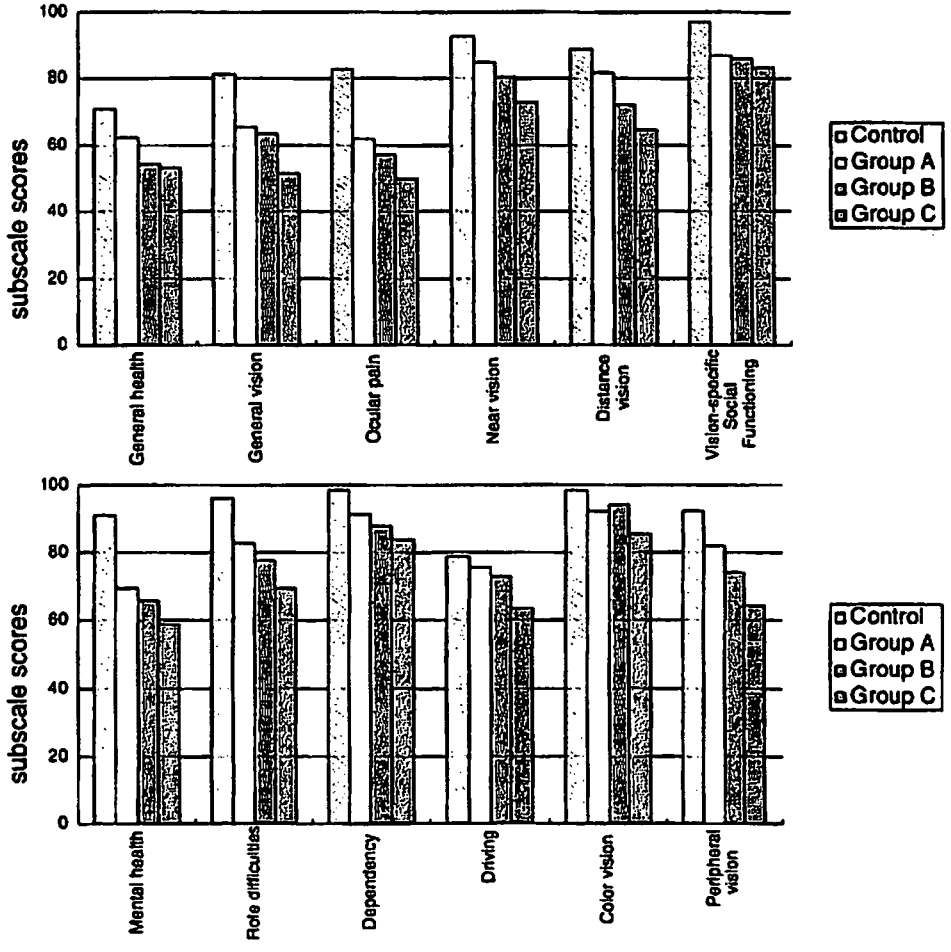

FIG. 1. Mean NEI-VFQ-25 subscale scores in groups A, B, and C and in control subjects. All group A, B, and C subscale scores (other than general health and driving in group A) were lower than those of age-matched control subjects ($P<0.05$, Mann–Whitney test).

patients with keratoconus. Possible explanations for the reduced VR-QOL observed in patients with keratoconus and normal measured visual acuity may include medical and biologic issues, such as changes in ocular surface characteristics resulting in tear film alterations,[19] which may render traditional measures of visual function incomplete in the examination of patients with keratoconus. Additionally, despite apparently normal visual function, patients with keratoconus may experience other disease-related burdens, such as contact lens intolerance, ocular pain caused by epithelial and stromal damage, the need to seek frequent ophthalmologic care, and anxiety related to disease progression and the possible need for surgery.

An important advantage of using generic instruments to measure VR-QOL, such as the NEI-VFQ-25 used in this study, is that such generic instruments facilitate the comparison of total and subscale scores among different groups of patients with various ocular disorders. When the results from the patients with keratoconus are compared with the findings from patients with other ocular disorders, such as uveitis, diabetic retinopathy, glaucoma, cataracts, and multiple sclerosis,[6-13,20,21] low scores in the ocular pain, general health, and mental health categories appear to be characteristic of patients with keratoconus.[16] The overall NEI-VFQ-25 profile of patients with keratoconus appears similar to that of patients with uveitis.[20] This may be partly related to the fact that both groups of patients experience ocular pain, frequently experience other chronic disorders beginning in young adulthood, require frequent ophthalmologic care, and fear progression to low vision.

The results of this study support the use of the NEI-VFQ-25 in patients with keratoconus and provide further evidence for the general applicability of this scale in various ocular disorders. The results underscore the importance of adopting a comprehensive approach to the examination and care of patients with keratoconus that incorporates but goes beyond measures of biologic disease status, such as visual acuity. Ophthalmologists and practitioners of other clinical specialties alike should be appraised of and bear in mind the full spectrum of QOL issues suffered by patients with keratoconus, such that the medical treatment that these patients receive may respond appropriately to the full range of issues these patients face.

REFERENCES

1. Feder RS. Noninflammatory ectatic disorders. In: Krachmer JH, Mannis MJ, Holland EJ, eds. *Cornea and External Disease: Clinical Diagnosis and Management*. St. Louis, Mosby–Year Book, 1997, pp 1091–1106.
2. Mangione CM, Berry S, Spritzer K, et al. Identifying the content area for the 51-item National Eye Institute Visual Function Questionnaire. *Arch Ophthalmol* 1998;116:227–233.
3. Schiffman RM, Christianson MD, Jacobsen G, et al. Reliability and validity of the ocular surface disease index. *Arch Ophthalmol* 2000; 118:615–621.
4. Steinberg EP, Tielsch JM, Schein LD, et al. The VF-14. *Arch Ophthalmol* 1994;112:630–638.
5. Paz SH, Globe DR, Wu J, et al. Los Angeles Latino Eye Study: Relationship between self-reported depression and self-reported visual function in Latinos. *Arch Ophthalmol* 2003;121:1021–1027.
6. Mangione CM, Lee PP, Gutierrez PR, et al. National Eye Institute Visual Function Questionnaire field test investigators. Development of the 25-item National Eye Institute Visual Function Questionnaire. *Arch Ophthalmol* 2001;119:1050–1508.
7. Deramo VA, Cox TA, Syed AB, et al. Vision-related quality of life in people with central retinal vein occlusion using the 25-item National Eye Institute Visual Function Questionnaire. *Arch Ophthalmol* 2003; 121:1297–1302.
8. Gutierrez P, Wilson R, Johnson C, et al. Influence of glaucomatous visual field loss on health-related quality of life. *Arch Ophthalmol* 1997;115:777–784.
9. Jampel HD, Schwartz A, Pollack I, et al. Glaucoma patients assessment of their visual function and quality of life. *J Glaucoma* 2002;11: 154–163.
10. Klein R, Moss SE, Klein BE, et al. The NEI-VFQ-25 in people with long-term type 1 diabetes mellitus. *Arch Ophthalmol* 2001;119:733–740.
11. Cahill MT, Banks AD, Stinnett SS, Toth CA. Vision-related quality of life in patients with bilateral severe age-related macular degeneration. *Ophthalmology* 2005;112:152–158.
12. Nichols KK, Mitchell GL, Zadnik K. Performance and repeatability of the NEI-VFQ-25 in patients with dry eye. *Cornea* 2002;21:578–583.
13. Felius J, Stager DR, Berry PM, et al. Development of an instrument to assess vision-related quality of life in young children. *Am J Ophthalmol.* 2004;138:362–372.
14. Suzukamo Y, Oshika T, Yuzawa M, et al. Psychometric properties of the 25-item National Eye Institute Visual Function Questionnaire (NEI VFQ-25), Japanese version. *Health Qual Life Outcomes* 2005;3:65.
15. Oshika T, Sugita G, Hayashi K, et al. Influence of cataract and intraocular lens surgery on health-related quality of life [in Japanese]. *Acta Soc Ophthalmol Jpn* 2005;109:753–760.
16. Kymes SM, Walline JJ, Zadnik K, et al. Collaborative Longitudinal Evaluation of Keratoconus (CLEK) Study Group: Quality of life in keratoconus. *Am J Ophthalmol* 2004;138:527–535.
17. Giedd KK, Mannis MJ, Mitchell GL, et al. Personality in keratoconus in a sample of patients derived from the Internet. *Cornea* 2005;24: 301–307.
18. Mannis MJ, Morrison TL, Zadnik K, et al. Personality trends in keratoconus: An analysis. *Arch Ophthalmol* 1987;105:798–800.
19. Dogru M, Karakaya H, Ozçetin H, et al. Tear function and ocular surface changes in keratoconus. *Ophthalmology* 2003;110:1110–1118.
20. Schiffman RM, Jacobsen G, Whitup SM. Visual functioning and general health status in patients with uveitis. *Arch Ophthalmol* 2001; 119:841–849.
21. Siu-Ling MA, Shea JA, Galetta SL, et al. Self-reported visual dysfunction in multiple sclerosis: New data from the VFQ-25 and development of an MS-specific vision questionnaire. *Am J Ophthalmol* 2002;133:686–692.

Deposition of Lipid, Protein, and Secretory Phospholipase A_2 on Hydrophilic Contact Lenses

Hiroshi Mochizuki, M.D., Masakazu Yamada, M.D., Shin Hatou, M.D., Motoko Kawashima, M.D., and Seiichiro Hata, M.D.

Eye & Contact Lens 34(1): 46–49, 2008

Deposition of Lipid, Protein, and Secretory Phospholipase A_2 on Hydrophilic Contact Lenses

Hiroshi Mochizuki, M.D., Masakazu Yamada, M.D., Shin Hatou, M.D., Motoko Kawashima, M.D., and Seiichiro Hata, M.D.

Purpose. Recent studies have shown that low tear phospholipid levels are associated with tear film instability in hydrophilic contact lens wearers. The concentration of secretory phospholipase A_2 ($sPLA_2$), the enzyme that hydrolyzes phospholipids, in tears is known to exceed the levels found in serum by four orders of magnitude. This study was performed to determine the levels of $sPLA_2$ from the deposition on two different frequent-replacement contact lens materials. **Methods.** Polymacon and etafilcon A contact lenses worn for 2 weeks by 16 experienced contact lens wearers were used for the analysis. Total lipids were determined by the sulfo-phospho-vanillin reaction. Phospholipids in lipid extracts were estimated by phosphorus determination with ammonium molybdate through enzymatic digestion. Total protein was measured by bicinchoninic acid analysis. Double-antibody sandwich enzyme-linked immunosorbent assay was used to determine $sPLA_2$ concentrations. **Results.** Total lipid deposition was found to be greater in the polymacon group (66.3 ± 16.3 μg/lens) than in the etafilcon A group, although phospholipids were not detected in either group. The etafilcon A group had greater deposition of protein (3.7 ± 0.7 mg/lens) than the polymacon group had. The etafilcon A group deposited statistically significantly more group IIa $sPLA_2$ (1.1 ± 0.3 μg /lens) than the polymacon group (0.07 ± 0.04 μg/lens) did ($P<0.001$). **Conclusions.** There was a significant difference in the lipid and protein deposition profiles in the two lenses tested. A significant amount of $sPLA_2$ in the deposition on contact lenses may play a role in tear film instability in hydrophilic contact lens wearers.

Key Words: Contact lens—Dry eye—Phospholipids—Secretory phospholipase A_2—Tears.

Contact lens–induced dry eye is one of the major causes of contact lens intolerance. Tear film stability, which is clinically estimated by tear film breakup time, is compromised in soft contact lens wearers,[1,2] especially in intolerant contact lens wearers.[3]

Recently, the structural and biologic roles of lipids in the tear film have been described in some detail.[4–6] The lipid layer is an essential component of the tear film by providing a smooth optical surface for the cornea and retarding evaporation from the ocular surface. In the current model of the tear film, the aqueous–mucin layer is covered by two thin layers consisting of lipids. Polar lipids, such as phospholipids, lie adjacent to the aqueous layer, and nonpolar lipids are present at the tear–air interface.[7] Recent studies have shown that the level of phospholipids in tears is the most influential factor for tear film stability, which is clinically estimated by tear film breakup time.[8] Thus, phospholipids, which link the nonpolar hydrophobic outer layer and the aqueous layer, are crucial for maintaining a stable tear film. A low level of phospholipids in the tears is associated with a short breakup time in hydrophilic contact lens wearers.[8–10]

Phospholipase A_2 (PLA_2) is a lipolytic enzyme that catalyzes the hydrolysis of phospholipids at the sn-2 position, yielding a free fatty acid and a lysophospholipid. PLA_2 has been categorized into at least 10 groups (I–X) based on amino acid sequence data.[11,12] Many of these enzymes are secreted extracellularly and are commonly referred to as secretory phospholipase A_2 ($sPLA_2$).[13] Of these, group IIa $sPLA_2$ (14 kilodaltons) is the most abundant form of $sPLA_2$ in tears.[14] It was found in concentrations in tears averaging 1.45 μg/mL[14] to 54.5 μg/mL,[15] which exceed the levels found in serum by four orders of magnitude. The presence of group IIa $sPLA_2$ in tears is supposed to be beneficial because of its bactericidal activity.[16,17] However, as suggested by Song et al.,[18] the excess of this enzyme may compromise the tear film stability, because it hydrolyzes phospholipids in tears.

As mentioned earlier, the level of phospholipids in tears of hydrophilic contact lens wearers is lower than that of healthy control subjects, which may be associated with instability of the tear film.[8–10] For the explanation of this phenomenon, two possibilities were hypothesized: Phospholipid deposition occurs on contact lenses, and phospholipids in tears are degraded by group IIa $sPLA_2$ deposited on contact lenses. To test these hypotheses, the levels of lipids, proteins, group IIa $sPLA_2$ content, and $sPLA_2$ activity were determined from the deposition on two different frequent-replacement hydrophilic contact lens materials.

From the Division for Vision Research (H.M., M.Y., S. Hatou), National Institute of Sensory Organs, National Tokyo Medical Center, Tokyo, Japan; the Department of Ophthalmology (M.K.), Tokyo Dental College Ichikawa General Hospital, Chiba, Japan; and Sky Building Eye Clinic (S. Hata), Yokohama, Japan.

Supported in part by a grant from Ministry of Health, Labor, and Welfare, Japan.

The authors have no proprietary interest in any aspect of this article.

Address correspondence and reprint requests to Dr. M. Yamada, Division for Vision Research, National Institute of Sensory Organs, National Tokyo Medical Center, 2-5-1 Higashigaoka, Meguro-ku, Tokyo 152-8902, Japan; e-mail: yamadamasakazu@kankakuki.go.jp

Accepted April 2, 2007.

DOI: 10.1097/ICL.0b013e3180676d5d

MATERIALS AND METHODS

Subjects and Contact Lenses

Sixteen experienced, asymptomatic contact lens wearers (six men and 10 women) ranging in age from 21 to 44 years participated in the study. Eight subjects were polymacon lens (Medalist; Bausch & Lomb Japan, Tokyo, Japan; Food and Drug Administration group I, low water content and nonionic) wearers, and eight subjects were etafilcon A lens (2-Week ACUVUE; Johnson & Johnson Japan, Tokyo, Japan; Food and Drug Administration group IV, high water content and ionic) wearers. Lenses worn for 2 weeks were collected and stored at −80°C until analysis. Each lens was cut in half for lipid and protein deposits analysis. All subjects provided written informed consent for participation. The protocol was approved by the institutional review board.

Lipid Analysis

Lipids were extracted by a modification of the Bligh and Dyer procedure.[19] In brief, samples were placed in a test tube with 1.0 mL of an extraction solvent consisting of a 2:1 ratio of chloroform to methanol (Wako, Inc., Osaka, Japan) for 16 hours. After adding 0.2 mL of water, test tubes were vortexed for 30 seconds. The upper aqueous layer was discarded, and the lower organic solvent layer was used for the analysis.

Total lipids were measured by the sulfo-phospho-vanillin reaction. The lipid extracts were evaporated to dryness under nitrogen gas and reconstituted with 50 μL of distilled water. After adding 100 μL of 95% sulfuric acid (Wako, Inc.), the sample was boiled at 100°C for 10 minutes. Then, samples placed in a 96-well microplate were mixed with 150 μL of the working reagent, containing 1.2 mg/mL vanillin (Kokusai Shiyaku Co., Tokyo, Japan). The absorbance of the solution was measured at 655 nm with a spectrophotometer. A standard curve established with lipids solution (Kokusai Shiyaku Co.) was used to quantify the total lipids of the lens extract.

Phospholipids were estimated by phosphorus determination with ammonium molybdate through enzymatic digestion. After lipid extracts were evaporated to dryness under nitrogen gas, 50 μL of 10 mM TRIS hydrochloride (Sigma Chemical Co., St. Louis, MO) buffer (pH 7.8), containing 2.0 U/mL phospholipase C from *Bacillus cereus* (Sigma Chemical Co.), was added and incubated at 37°C for 20 minutes. The samples were incubated at 37°C for an additional 30 minutes after adding 50 μL of 175 mM diethanolamine hydrochloride (Sigma Chemical Co.) buffer (pH 9.6), containing 2.0 U/mL alkaline phosphatase from human placenta (Sigma Chemical Co.). Fifty microliters of samples were placed in a 96-well microplate and mixed with molybdate–malachite green reagent (BIOMOL, Inc., Plymouth Meeting, PA). The absorbance of the solution was measured at 620 nm with a spectrophotometer. A standard curve established with phospholipids mixture (Sigma Chemical Co.) was used to quantify the amounts of phospholipids.

Total Protein and $sPLA_2$ Analysis

A solvent consisting of a 1:1 mixture of 0.2% trifluoroacetic acid and acetonitrile (Wako, Inc.) was used to extract protein.[20] The lenses were placed in the extraction solution for 16 hours, and the extraction solution was subsequently analyzed. This method is a quick, efficient extraction technique for the removal of protein deposits from soft hydrophilic contact lenses. Determination of the total protein deposit from the lenses was carried out by a bicinchoninic acid analysis. The procedure consists of mixing 10 μL of sample solution with 300 μL of protein assay regent (Cytoskeleton, Inc., Denver, CO) comprising bicinchoninic acid and cupric sulfate in a 96-well microplate. Absorbance of the solution was measured at 595 nm with a spectrophotometer. A standard curve established with bovine serum (Sigma Chemical Co.) was used to quantify protein contents of the lens extract.

A double-antibody sandwich enzyme-linked immunosorbent assay was used to determine group IIa $sPLA_2$ concentrations in the protein samples. A commercial enzyme-linked immunosorbent assay kit (Cayman Chemicals, Ann Arbor, MI) was used according to the manufacturer's instructions. Samples were diluted to 1/500 or 1/5,000 concentrations. Absorbance was measured at 420 nm with a spectrophotometer.

Measurement of $sPLA_2$ activity was performed with a commercial $sPLA_2$ activity assay kit (Cayman Chemicals). The assay uses a 1,2-dithio analog of heptanoyl phosphatidylcholine, which serves as a substrate for most PLA_2, with the exception of cytosolic PLA_2. On hydrolysis of the thioester bond at the sn-2 position by PLA_2, free thiols were detected by using 5,5-dithio-bis(2-nitorobenzonic acid). Absorbance was measured at 420 nm with a spectrophotometer.

RESULTS

Total Lipids and Phospholipids

Total lipids and phospholipids deposition are shown in Figure 1. Total lipids were found to be greater in the polymacon group (66.3 ± 16.3 μg/lens) than in the etafilcon A group (44.1 ± 8.2 μg/lens). The difference between the two groups was statistically significant ($P<0.01$, Mann–Whitney test). Phospholipids were not detected in either group.

Total Protein

Results of total protein deposition analysis are shown in Figure 2. The etafilcon A group (3.7 ± 0.7 mg/lens) deposited substantially more protein than the polymacon group (0.03 ± 0.06 mg/lens) did. The difference was statistically significant ($P<0.001$, Mann–Whitney test).

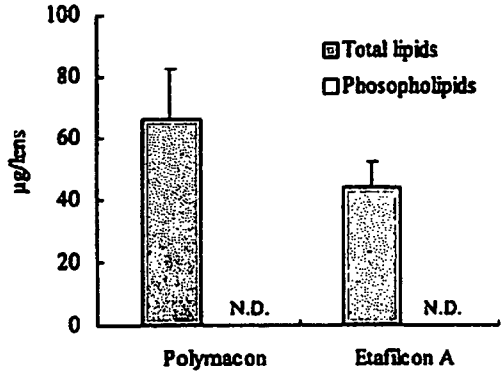

FIG. 1. Total lipids and phospholipids deposited on hydrophilic contact lenses. Total lipids were significantly greater in the polymacon group (66.3 ± 16.3 μg/lens) than in the etafilcon A group (44.1 ± 8.2 μg/lens). Phospholipids were not detected in either group.

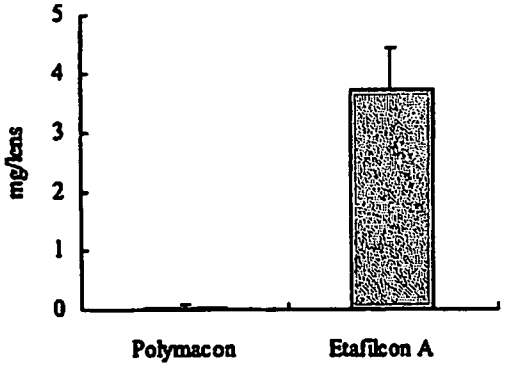

FIG. 2. Total proteins deposited on hydrophilic contact lenses. The etafilcon A group (3.7 ± 0.7 mg/lens) deposited significantly more protein than the polymacon group (0.03 ± 0.06 mg/lens) did.

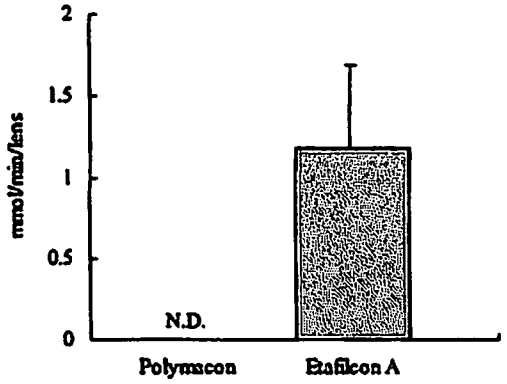

FIG. 4. $sPLA_2$ activity in the etafilcon A group was 1.18 ± 0.59 mmol/minute per lens, whereas $sPLA_2$ activity was not detected in the polymacon group.

Group IIa $sPLA_2$ and $sPLA_2$ Activity

The amount of group IIa $sPLA_2$ and its enzymatic activity deposited on contact lenses are shown in Figures 3 and 4. The etafilcon A group (1.1 ± 0.3 μg /lens) deposited more group IIa $sPLA_2$ than the polymacon group (0.07 ± 0.04 μg/lens) did. The difference was statistically significant ($P<0.001$, Mann–Whitney test). $sPLA_2$ deposited on the etafilcon A lenses retained its enzymatic activity. $sPLA_2$ activity in the etafilcon A group was 1.18 ± 0.59 mmol/minute per lens. In the polymacon group, $sPLA_2$ activity was not detected.

DISCUSSION

It is widely recognized that the adsorption of proteins and lipids on a contact lens is complex and depends on a number of factors. Notable among these are material water content and surface charge.[21–24] There was a significant difference in the lipid and protein deposition profiles between the two lenses tested in the current study. The lipid deposition profiles found in this study are consistent with previously published reports,[23,25] which concluded that hydrogel lenses with nonionic polymer matrices (i.e., groups I and II) deposit more lipids than materials that have ionic matrices (i.e., groups III and IV).[26] In the current study, however, phospholipid depositions were below the detection limit in either lens. Therefore, it is not likely that decreased phospholipid levels in tears of hydrophilic contact lens wearers is the result of the deposition of phospholipids on the lenses.

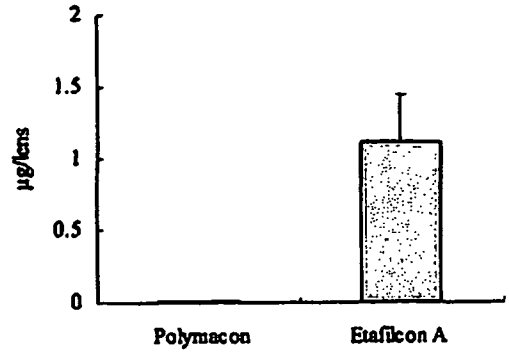

FIG. 3. Group IIa sPLA2 deposited on hydrophilic contact lenses. The etafilcon A group (1.1 ± 0.3 μg /lens) deposited significantly more group IIa $sPLA_2$ than the polymacon group (0.07 ± 0.04 μg/lens) did.

Etafilcon A (group IV material) attracted substantial quantities of protein, which was significantly greater than that measured for polymacon (group I material). Etafilcon A also had significantly more group IIa $sPLA_2$ than polymacon did. Protein deposition was predominantly controlled by the ionic charge of the lens material.[25] Methacrylic acid imparts a negative charge to the material and thus thermodynamically favors the deposition of positively charged species, such as lysozymes. Group IIa $sPLA_2$ is also highly cationic in tears[27] and allows productive electrostatic interactions with the negatively charged contact lens material.

It appears to be important that $sPLA_2$ deposited on etafilcon A lenses retained its enzymatic activity.[8,10] Hume et al.[17] reported that $sPLA_2$ deposited on contact lenses reduced the viable staphylococci adhering to the contact lens, which may be beneficial for the eye to prevent colonization by this pathogen. However, as suggested by Song et al.,[18] the excess of $sPLA_2$ may compromise tear film stability, which results in contact lens intolerance. Aho et al.[28] reported that contact lens wearers had statistically lower group IIa sPLA2 content in their tears at noon and at 4 P.M. than healthy control subjects did. They pointed out that the transient lowering effect on the group IIa sPLA2 content of tears may be the result of the absorption of group IIa sPLA2 onto the contact lenses. The current results appear to support these previous observations. Glasson et al.[29] reported that intolerant contact lens wearers had significantly greater concentration of group IIa $sPLA_2$ and peroxidized lipids in their tear fluids than tolerant subjects did. They suggested that decreased tear phospholipids and tear film instability may be the result of the action of group IIa $sPLA_2$ in the tear fluids. The current study found that a significant amount of group IIa $sPLA_2$ (1.1 ± 0.3 μg/lens) deposited on etafilcon A contact lenses. By assuming that the total tear volume of a healthy subject is 10 μL and that the concentration of group IIa $sPLA_2$ in normal tear fluids is 54.5 μg/mL,[15] 0.55 μg of group IIa $sPLA_2$ is present in the tears of healthy subjects. The amount of group IIa $sPLA_2$ deposited on etafilcon A contact lens is twice that in normal tear fluids. These results suggest an additional mechanism of contact lens–induced dry eye; group IIa $sPLA_2$ deposited on contact lenses may play a role in the development of tear film instability.

In summary, this study found that a significant amount of group IIa $sPLA_2$ deposited on contact lenses, at least on etafilcon A lenses. The enzyme deposited on contact lenses may promote

phospholipid hydrolysis in the tear fluids, which results in a decrease of phospholipids and an increase of free fatty acids in tears. These biochemical alterations in tears could cause tear film instability and may be associated with discomfort during contact lens wear.

REFERENCES

1. Faber E, Golding TR, Lowe R, et al. Effect of hydrogel lens wear on tear film stability. *Optom Vis Sci* 1991;68:380–384.
2. Guillon JP, Guillon M. Tear film examination of the contact lens patient. *Optician* 1993;206:21–29.
3. Glasson MJ, Stapleton F, Keay L, et al. Differences in clinical parameters and tear film of tolerant and intolerant contact lens wearers. *Invest Ophthalmol Vis Sci* 2003;44:5116–5124.
4. McCulley JP, Shine W. A compositional based model for the tear film lipid layer. *Trans Am Ophthalmol Soc* 1997;95:79–88.
5. Bron AJ, Tiffany JM. The meibomian glands and tear film lipids. Structure, function, and control. *Adv Exp Med Biol* 1998;438:281–295.
6. Greiner JV, Glonek T, Korb DR, et al. Meibomian gland phospholipids. *Curr Eye Res* 1996;15:371–375.
7. Korb DR, Greiner JV, Glonek T. Tear film lipid layer formation: Implications for contact lens wear. *Optom Vis Sci* 1996;73:189–192.
8. Guillon M, Maissa C, Girard-Claudon K, et al. Influence of the tear film composition on tear film structure and symptomatology of soft contact lens wearers. *Adv Exp Med Biol* 2002;506:895–899.
9. Kawashima M, Yamada M, Arita R, et al. Measurement of phospholipids in human tears [in Japanese]. *Folia Ophthalmol Jpn* 2003;54: 870–873.
10. Yamada M, Mochizuki H, Kawashima M, et al. Phospholipids and their degrading enzyme in the tears of soft contact lens wearers. *Cornea* 2006;25:S68–S72.
11. Cupillard L, Koumanov K, Mattei MG, et al. Cloning, chromosomal mapping, and expression of a novel human secretory phospholipase A_2. *J Biol Chem* 1997;272:15745–15752.
12. Dennis EA. The growing phospholipase A_2 superfamily of signal transduction enzymes. *Trends Biochem Sci* 1997;22:1–2.
13. Waite M. Phospholipases, enzymes that share a substrate class. *Adv Exp Med Biol* 1990;279:1–22.
14. Nevalainen TJ, Aho HJ, Peuravuori H. Secretion of group 2 phospholipase A_2 by lacrimal glands. *Invest Ophthalmol Vis Sci* 1994;35:417–421.
15. Saari KM, Aho V, Paavilainen V, et al. Group II PLA(2) content of tears in normal subjects. *Invest Ophthalmol Vis Sci* 2001;42:318–320.
16. Moreau JM, Girgis DO, Hume EBH, et al. Phospholipase A_2 in rabbit tears: A host defense against *Staphylococcus aureus*. *Invest Ophthalmol Vis Sci* 2001;42:2347–2354.
17. Hume EBH, Cole N, Parmar A, et al. Secretory phospholipase A_2 deposition on contact lenses and its effect on bacterial adhesion. *Invest Ophthalmol Vis Sci* 2004;45:3161–3164.
18. Song CH, Choi JS, Kim DK, et al. Enhanced secretory group II PLA_2 activity in the tears of chronic blepharitis patients. *Invest Ophthalmol Vis Sci* 1999;40:2744–2748.
19. Lapetina EG, Schmitges CJ, Chandrabose K, et al. Cyclic adenosine 3',5'-monophosphate and prostacyclin inhibit membrane phospholipase activity in platelets. *Biochem Biophys Res Commun* 1977;76: 828–835.
20. Keith D, Hong B, Christensen M. A novel procedure for the extraction of protein deposits from soft hydrophilic contact lenses for analysis. *Curr Eye Res* 1997;16:503–510.
21. Minarik L, Rapp J. Protein deposits on individual hydrophilic contact lenses: Effects of water and ionicity. *CLAO J* 1989;15:185–188.
22. Minno GE, Eckel L, Groemminger S, et al. Quantitative analysis of protein deposits on hydrophilic soft contact lenses: I. Comparison to visual methods of analysis. II. Deposit variation among FDA lens material groups. *Optom Vis Sci* 1991;68:865–872.
23. Maissa C, Franklin V, Guillon M, et al. Influence of contact lens material surface characteristics and replacement frequency on protein and lipid deposition. *Optom Vis Sci* 1998;75:697–705.
24. Fowler SA, Korb DR, Allansmith MR. Deposits on soft contact lenses of various water contents. *CLAO J* 1985;11:124–127.
25. Bontempo AR, Rapp J. Protein-lipid interaction on the surface of a rigid gas-permeable contact lens in vitro. *Curr Eye Res* 1997;16:1258–1262.
26. Jones L, Evans K, Sariri R, et al. Lipid and protein deposition of N-vinyl pyrrolidone-containing group II and group IV frequent replacement contact lenses. *CLAO J* 1997;23:122–126.
27. Scott DL, Mandel AM, Sigler PB, et al. The electrostatic basis for the interfacial binding of secretory phospholipases A_2. *Biophys J* 1994; 67:493–504.
28. Aho VV, Paavilainen V, Nevalainen TJ, et al. Diurnal variation in group IIa phospholipase A_2 content in tears of contact lens wearers and normal controls. *Graefes Arch Clin Exp Ophthalmol* 2003;241:85–88.
29. Glasson M, Stapleton F, Willcox M. Lipid, lipase and lipocalin differences between tolerant and intolerant contact lens wearers. *Curr Eye Res* 2002;25:227–235.

Fluorophotometric measurement of the precorneal residence time of topically applied hyaluronic acid

H Mochizuki,[1] M Yamada,[1] S Hato,[1] T Nishida[2]

[1] Division for Vision Research, National Institute of Sensory Organs, National Tokyo Medical Center, Tokyo, Japan; [2] Department of Ophthalmology, Yamaguchi University Graduate School of Medicine, Yamaguchi, Japan

Correspondence to: M Yamada, Division for Vision Research, National Institute of Sensory Organs, National Tokyo Medical Center, 2-5-1 Higashigaoka, Meguro, Tokyo 152-8902, Japan; yamadamasakazu@kankakuki.go.jp

Accepted 6 June 2007

ABSTRACT

Purpose: This study was performed to separately assess the aqueous flow applied with hyaluronic acid, and the behaviour of hyaluronic acid itself on the ocular surface.

Methods: Two different fluorescent dyes, fluorescein sodium dissolved in 0.1% hyaluronic acid (HA) solution and 0.1% fluorescein conjugated with hyaluronic acid (F-HA) dissolved in saline, were used. A volume of 20 μl of tested solution was applied to the eye of 10 healthy volunteers. Fluorescein sodium dissolved in saline served as a control. The fluorescent intensity of the precorneal tear film was measured at the central cornea every minute for 10 min. The turnover rate was calculated using the equation that plots fluorescent intensity against time in a semilog plot and expressed as %/min.

Results: Turnover rates of topically applied 0.1% F-HA, 0.1% HA and saline were 8.1 (SD 3.6)%/min, 21.6 (2.8)%/min, and 31.0 (3.7)%/min, respectively. The turnover rate of F-HA was significantly lower than those of HA and saline ($p = 0.00012$ and $p = 0.00000022$, respectively; Mann–Whitney test). The turnover rate of HA was significantly lower than that of saline ($p = 0.00001$; Mann–Whitney test).

Conclusion: Our results indicate that the bulk aqueous flow applied with HA and the turnover of HA itself are different. HA molecules may adhere to the ocular surface by surface-chemical and/or biochemical properties. The long retention time of HA on the ocular surface may explain the mechanism in which hyaluronic acid has been shown to enhance tear film stability for a few hours.

Dry eye is a common condition, affecting approximately 10–20% of the adult population.[1] The clinical consequences of dry eye may include symptomatic irritation, superficial punctuate keratopathy, corneal erosions and possibly visual acuity problems.[2] Dry eye is considered to be a disorder of the tear film due to tear deficiency or excessive evaporation, which causes damage to the interpalpebral ocular surface and is associated with symptoms of ocular discomfort.[3]

A variety of treatment modalities have been used for the treatment of dry eye. The majority of these fall into the category of tear substitutes or replacements.[4] Artificial tears, the most frequently used modality for the treatment of dry eye, may be effective in relieving symptoms in mild dry eye by replenishing deficient tear volume. However, in moderate and severe cases of dry eye, artificial tears alone are not enough to relieve the symptoms nor to improve superficial punctuate keratopathy.[4]

Since preliminary reports in the early 1980s,[5–7] several studies have reported that hyaluronic acid is able to improve the symptoms, signs and ocular surface damage associated with dry eye syndrome.[8–11] Hyaluronic acid is a glycosaminoglycan with a viscoelastic rheology. Its relatively high viscosity is believed to improve tear-film stability and to reduce washout from the ocular surface.[12] Hyaluronic acid enhances water retention on the corneal surface, and probably increases corneal wettability.[13] In addition, hyaluronic acid promotes migration of corneal epithelial cells and accelerates the healing of corneal epithelial defects.[14–16] Hyaluronic acid has thus become an important treatment modality for dry eye.

Conflicting results, however, have been obtained regarding the duration of the action of hyaluronic acid on the ocular surface. The residence time of topically applied hyaluronic acid assessed by the tear meniscus height and water evaporation rate from the ocular surface was less than 10 min, although this was significantly longer than that of phosphate-buffered saline.[17 18]

Using an assessment of the tear-film breakup time, however, hyaluronic acid has been shown to enhance tear-film stability for more than a few hours.[19–21] These observations suggest that hyaluronic acid remains on the ocular surface independent of the bulk aqueous flow.

In order to test this hypothesis, we used two different fluorescent dyes, fluorescein sodium dissolved in hyaluronic acid solution and fluorescein conjugated with hyaluronic acid dissolved in saline, in the current study. The former is a well-established dye used to assess the bulk aqueous flow, and the latter dye is used as a tracer to determine the behaviour of hyaluronic acid on the ocular surface. Although the residence time of topically applied hyaluronic acid has been investigated using $^{99}Tc^{m}$ as a tracer,[22–24] we believe that this study is the first report to measure the residence time using a tracer that is associated with hyaluronic acid on the ocular surface.

SUBJECTS AND METHODS

Fluorescent dye and fluorophotometer

Fluorescein hyaluronic acid (F-HA, Mw; 800 000 Da) was purchased from Sigma-Aldrich (St. Louis, MO). F-HA, fluorescein conjugated with hyaluronic acid, was dissolved in a phosphate-buffered saline as 0.1% solution and used as a tracer of hyaluronic acid. Fluorescein sodium (0.001%; Sigma-Aldrich) and 0.1% hyaluronic acid (Mw; 800 000 Da, Sigma-Aldrich) dissolved in a phosphate-buffered saline was used as a tracer of the bulk aqueous flow in the presence of hayaluronic acid. Fluorescein sodium (0.001%) in a phosphate-buffered saline was used as a tracer of the bulk aqueous flow.

A commercial slit-lamp fluorophotometer (Anterior Fluorometer FL-500, Kowa Co., Tokyo) was used. The illuminating light was focused as a 2-mm diameter circle on the surface of the cornea. The emitted light passed through a band-interference filter centred on 565 nm (half bandwidth 25 nm) and was directed to a photomultiplier tube with the band-interference filter centred on wavelengths 490 nm (half bandwidth 30 nm).

F-HA solution (0.5%) was diluted in a phosphate-buffered saline to produce sets of standards ranging from 0.001% to 0.5% in concentration for the calibration. A cuvette was constructed by gluing together two microscope slides and two cover glasses. The cover glasses were sandwiched by two microscope slides in order to provide space for the fluid layer to be 12–15 μm thick. A fresh one was made for each solution. Ten microlitres of calibrating fluids, containing 0.001–0.5% F-HA, was placed into a cuvette. The fluorescent intensity was measured by a slit-lamp fluorophotometer. The interaction of F-HA with the proteins was also tested using a phosphate-buffered saline containing 1% fetal bovine serum.

Measurement of residence time

Ten healthy volunteers (five male and five female) aged 27–44 years (33.8 (SD 6.8) years old, mean (SD)), who had no history of eye disease, except for refractive errors, were enrolled in this study. The principles of the World Medical Association Declaration of Helsinki were followed. The subjects received a full explanation of the procedures, and provided their informed consent for participation prior to the experiment. The protocol was approved by our institutional review board, and all subjects provided their written informed consent.

In our experiments, the subjects were seated in front of the fluorophotometer. The instrument was focused on the central cornea, and the background fluorescent intensity was measured. A volume of 20 μl of tested solution was applied to the eye with an Eppendorf micropipette without making contact. The subjects were then instructed to blink several times to ensure the mixing of the dye. The fluorescent intensity of the precorneal tear film was measured at the central cornea every minute for 10 min. Repeated measurements on different days were made in some subjects to evaluate the repeatability of the test.

The turnover rate is given by the following equation, which plots fluorescent intensity against time in a semilog plot:

$$F = F_0 \exp(-kt)$$

where F is the fluorescent intensity at time (t); F_0 is the fluorescent intensity at time zero; k is the turnover rate; and t is the time in minutes.[25] The turnover rate was calculated using the equation and expressed as %/min. The regression fit of the log of the fluorescent intensity was recorded as the regression coefficient.

In all cases of 0.1% F-HA and 0.1% hyaluronic acid, this regression became a straight line. In cases of saline, however, this regression sometimes showed a biphasic response: an initial faster and a subsequent lower turnover rate. When the turnover rate of saline became biphasic, the subsequent lower turnover rate was used as the flow rate of saline.[25]

All results are presented as the mean ± 1 standard deviation (SD). Statistical significance was calculated by comparing results using the Mann–Whitney test. A value of $p<0.05$ was considered to indicate statistical significance.

RESULTS

Calibration of F-HA

The calibration of the fluorescent intensities against the concentrations of 0.001–0.5% F-HA is shown in fig 1. The relationship between the fluorescent intensities and the concentrations of F-HA was linear ($r^2 = 0.995$). The data generated by this method were consistent and reproducible. The fluorescent intensities of F-HA were unaffected by the presence of 1% fetal bovine serum (data not shown).

Turnover-rate measurements

A typical result of turnover-rate measurements obtained from one subject is shown in fig 2. In the presented case, the fluorescent intensities of 0.1% F-HA decayed with time at a flow rate of 7.6%/min, which was lower than those of fluorescein sodium in 0.1% hyaluronic acid (19.4%/min) and in saline (28.1%/min).

The turnover rates of topically applied F-HA, hyaluronic acid and saline obtained from 10 subjects were 8.1 (3.6)%/min, 21.6 (2.8)%/min, and 31.0 (3.7)%/min, respectively (table 1). The turnover rate of F-HA was significantly lower than those of hyaluronic acid and saline ($p = 0.00012$, and $p = 0.00000022$, respectively; Mann–Whitney test). The turnover rate of F-HA was significantly lower than that of saline ($p = 0.00001$; Mann–Whitney test).

DISCUSSION

In the current study, we used two different fluorescent dyes, fluorescein sodium dissolved in hyaluronic acid solution and F-HA solution, to separately assess the aqueous flow applied with hyaluronic acid, and the behaviour of hyaluronic acid itself on the ocular surface. Our results indicate that there are two different aspects of the duration of topically applied hyaluronic acid.

Hyaluronic acid has a high-molecular-weight, naturally occurring glycosaminoglycan. Its relatively high viscosity is believed to reduce washout from the ocular surface.[13] The residence time of topically applied hyaluronic acid has previously been investigated by using gamma scintigraphic methods.[22–24] Snibson and associates[22 24] reported that hyaluronic acid had prolonged ocular residence times in comparison

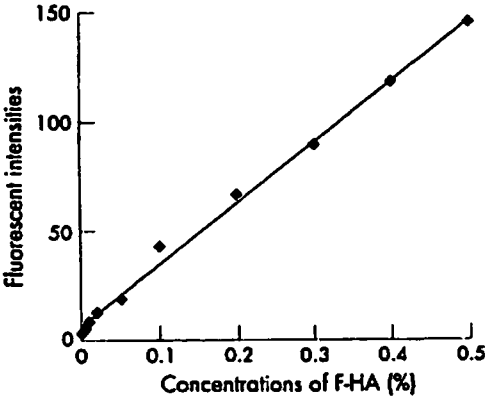

Figure 1 Calibration of the fluorescent intensities against the concentrations of flurorescein hyaluronic acid (F-HA). The relationship between the fluorescent intensities and the concentrations of F-HA was linear ($r^2 = 0.995$).

Table 1 Turnover rates of topically applied 0.1% fluorescein hyaluronic acid (F-HA), 0.1% hyaluronic acid and saline obtained from 10 subjects

Subject no.	Turnover rate (%/min)		
	F-HA	Hyaluronic acid	Saline
1	7.6	19.4	28.1
2	12.2	22.6	31.0
3	2.9	22.6	31.8
4	9.5	20.4	33.2
5	4.6	21.4	33.4
6	8.6	22.2	37.1
7	15.2	24.2	29.6
8	8.4	20.4	24.1
9	5.3	17.1	27.8
10	7.1	21.3	33.9
Mean (SD)	8.1 (3.6)	21.7 (2.8)	31.0 (3.7)

The turnover rate of F-HA was significantly lower than those of hyaluronic acid and saline ($p = 0.00012$ and $p = 0.00000022$, respectively; Mann–Whitney test).

with a buffered saline solution, a solution containing polyvinyl alcohol or hydroxypropylmethylcellulose. In the current study, the turnover rate of the 0.1% hyaluronic acid solution (21.6 (2.8)%/min) was significantly lower than that of the saline (31.0 (3.7)%/min). Our result is considered to be in good accordance with the previous studies using scintigraphic methods.[22–24] This effect, however, appears to be transient, because 90% of the hyaluronic acid solution was calculated to be cleared from the ocular surface 10.7 min after instillation. This result is also consistent with the duration of topically applied hyaluronic acid assessed by the tear meniscus height and water evaporation rate from the ocular surface.[17 18]

The most interesting finding of the current study is that the turnover rate of F-HA (8.1 (3.6)%/min) was approximately one-third of the 0.1% hyaluronic acid solution (21.6 (2.8)%/min). This result indicates that the bulk aqueous flow applied with hyaluronic acid and the turnover of hyaluronic acid itself on the ocular surface are different. Besides viscosity, hyaluronic acid molecules may adhere to the ocular surface by surface-chemical and/or biochemical properties, because hyaluronic acid is known to bind with fibronectin and CD44, a cell surface adhesion molecule which has been found on corneal epithelial cells.[16 26] Snibson and associates[24] made a similar consideration based on their scintigraphic results. They, however, also mentioned the limitation of their methodology and the necessity of tracers that directly associate with hyaluronic acid. We believe that F-HA is a useful tracer to determine the behaviour of topically applied hyaluronic acid. According to our results, the times for 90% and 99% of hyaluronic acid to be cleared from the ocular surface were calculated to be 28.8 min and 57.6 min after instillation, respectively. The long retention time of hyaluronic acid in the ocular surface may explain the fact that hyaluronic acid has been shown to enhance tear-film stability for more than a few hours.[19–21]

Besides its biological effects on the corneal epithelial cells, hyaluronic acid appears to have two beneficial effects for the treatment of dry eye syndrome. First, it reduces the bulk aqueous flow by its viscosity and increases tear volume for a limited time, as do other viscous agents, such as chondroitin sulfate, polyvinyl alcohol and hydroxypropylmethylcellulose.[22] Second, hyaluronic acid remains on the ocular surface for a longer time, in order to increase corneal wettability and to retain tear fluid on the corneal surface.[12 13] This effect may be unique for hyaluronic acid, although it should be confirmed by further investigations.

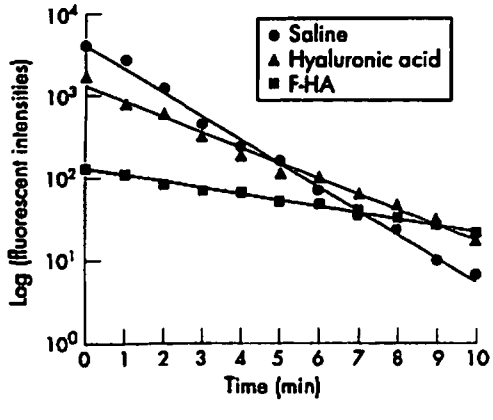

Figure 2 Typical result of the turnover rate measurements obtained from one subject. In the presented case, the fluorescent intensities of 0.1% flurorescein hyaluronic acid (F-HA) decayed with time at a flow rate of 7.6%/min, which was lower than those of fluorescein sodium in 0.1% hyaluronic acid (19.4%/min) and in saline (28.1%/min).

Competing interests: The authors have no proprietary interest in any materials in this manuscript.

REFERENCES

1. **Johnson ME,** Murphy PJ. Changes in the tear film and ocular surface from dry eye syndrome. *Prog Retin Eye Res* 2004;**23**:449–74.
2. **Holy FJ,** Lemp MA. Tear physiology and dry eyes. *Surv Ophthalmol* 1977;**22**:69–87.
3. **Lemp MA.** Report of the National Eye Institute/Industry workshop on clinical trials in dry eyes. *CLAO J* 1995;**21**:221–32.
4. **Nelson JD,** Farris RL. Sodium hyaluronate and polyvinyl alcohol artificial tear preparations. *Arch Ophthalmol* 1988;**106**:484–7.
5. **Polack FM,** McNiece MT. The treatment of dry eyes with Na hyaluronate (Healon): a preliminary report. *Cornea* 1982;**1**:133–6.
6. **Stuart JC,** Linn JG. Dilute sodium hyaluronate (Healon) in the treatment of ocular surface disorders. *Ann Ophthalmol* 1985;**17**:190–2.
7. **DeLuise VP,** Peterson WS. The use of topical Healon tears in the management of refractory dry-eye syndrome. *Ann Ophthalmol* 1984;**16**:823–4.
8. **Aragona P,** Papa V, Micali A, *et al.* Long term treatment with sodium hyaluronate-containing artificial tears reduces ocular surface damage in patients with dry eye. *Br J Ophthalmol* 2002;**86**:181–4.
9. **Limberg MB,** McCaa C, Kissling GE, *et al.* Topical application of hyaluronic acid and chondroitin sulfate in the treatment of dry eyes. *Am J Ophthalmol* 1987;**103**:194–7.
10. **Shimmura S,** Ono M, Shinozaki K, *et al.* Sodium hyaluronate eye drops in the treatment of dry eyes. *Br J Ophthalmol* 1995;**70**:1007–11.
11. **Condon PI,** McEwen CG, Wright M, *et al.* Double blind, randomized, placebo controlled, crossover, multicentre study to determine the efficacy of a 0.1% (w/v) sodium hyaluronate solution (Fermavisc) in the treatment of dry eye syndrome. *Br J Ophthalmol* 1999;**83**:1121–4.
12. **Nakamura S,** Okada S, Umeda Y, *et al.* Development of a rabbit model of tear film instability and evaluation of viscosity of artificial tear preparations. *Cornea* 2004;**23**:390–7.
13. **Nakamura M.** Hikida M, Nakano T, *et al.* Characterization of water retentive properties of hyaluronan. *Cornea* 1993;**12**:433–6.
14. **Nishida T,** Nakamura M, Mishima H, *et al.* Hyaluronan stimulates corneal epithelial migration. *Exp Eye Res* 1991;**53**:753–8.
15. **Sugiyama T,** Miyauchi S, Machida A, *et al.* The effect of sodium hyaluronate on the migration of rabbit corneal epithelium. The effect of topical administration. *J Ocular Pharmacol* 1991;**7**:53–64.

16. **Gomes JAP,** Amankwah R, Powell-Richards A, *et al*. Sodium hyaluronate promotes migration of human corneal epithelial cells in vitro. *Br J Ophthalmol* 2004;88:821–5.
17. **Watanabe T,** Kawashima M, Kawai M, *et al*. Effect of sodium hyaluronate eyedrops on tear meniscus height determined by fluorescein photography. [in Japanese] *Folia Ophthalmol Jpn* 2004;55:369–73.
18. **Tsubota K,** Yamada M. Tear evaporation from the ocular surface. *Invest Ophthalmol Vis Sci* 1992;33:2942–50.
19. **Mengher LS,** Pandher KS, Bron AJ, *et al*. The effect of sodium hyaluronate (0.1%) on break-up time (NIBUT) in patients with dry eyes. *Br J Ophthalmol* 1986;70:442–7.
20. **Hamano T,** Horimoto K, Lee M, *et al*. Evaluation of the effect of sodium hyaluronate ophthalmic solution on tear film stability. [in Japanese] *J Jpn Ophthalmol Soc* 1993;97:928–32.
21. **Johnson ME,** Murphy PJ, Boulton M. Effectiveness of sodium hyaluronate eyedrops in the treatment of dry eye. *Graefe's Arch Clin Exp Ophthalmol* 2006;244:109–12.
22. **Snibson GR,** Greaves JL, Soper NDW, *et al*. Precorneal residence times of sodium hyaluronate solutions studied by quantitative gamma scintigraphy. *Eye* 1990;4:594–602.
23. **Gurny R,** Ryser JE, Tabatabay C, *et al*. Precorneal residence time in humans of sodium hyaluronate as measured by gamma scintigraphy. *Graefe's Arch Clin Exp Ophthalmol* 1990;228:510–2
24. **Snibson GR,** Greaves JL, Soper NDW, *et al*. Ocular surface residence times of artificial tear solutions. *Cornea* 1992;11:288–93.
25. **Mishima S,** Gasset A, Klyce Jr SD, *et al*. Determination of tear volume and tear flow. *Invest Ophthalmol Vis Sci* 1966;5:264–76.
26. **Asari A,** Morita M, Sekiguchi T, *et al*. Hyaluronan, CD44 and fibronectin in rabbit corneal wound healing. *Jpn J Ophthalmol* 1996;40:18–25.

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Printed in the UK by Henry Ling Limited, at The Dorset Press, Dorchester DT1 1HD HL/BJO/42/08

Eye & Contact Lens 34(2): 109–112, 2008

In Vitro Susceptibilities of Bacterial Isolates From Conjunctival Flora to Gatifloxacin, Levofloxacin, Tosufloxacin, and Moxifloxacin

Masakazu Yamada, M.D., Shin Hatou, M.D., and JunkoYoshida, M.D.

Purpose. To evaluate and compare the in vitro susceptibilities of various fluoroquinolones (i.e., gatifloxacin, levofloxacin, tosufloxacin, and moxifloxacin) against conjunctival bacterial flora. **Methods.** Two hundred sixty-six bacterial isolates were collected from the conjunctival sacs of 251 eyes of 224 patients (118 females and 106 males) ranging in age from 6 to 91 years old, who were scheduled for intraocular surgery at National Tokyo Medical Center. The minimum inhibitory concentration (MIC) was determined by broth dilution testing. **Results.** Of 266 isolates, 258 (97.0%) strains were gram-positive bacteria and eight (3.0%) strains were gram-negative bacteria. The MIC_{90} values of gatifloxacin, levofloxacin, tosufloxacin, and moxifloxacin against α-hemolytic streptococci were 0.39 μg/mL, 1.56 μg/mL, 0.39 μg/mL, and 0.20 μg/mL, respectively. The MIC_{90} values of gatifloxacin, levofloxacin, tosufloxacin, and moxifloxacin against *Staphylococcus aureus* were 1.56 μg/mL, 3.13 μg/mL, 0.78 μg/mL, and 0.78 μg/mL, respectively. The MIC_{90} values of gatifloxacin, levofloxacin, tosufloxacin, and moxifloxacin against *Staphylococcus epidermidis* were 1.56 μg/mL, 3.13 μg/mL, 3.13 μg/mL, and 0.78 μg/mL, respectively. **Conclusions.** Although the clinical usefulness and efficacy of newer fluoroquinolones remains to be defined by clinical outcomes, the current study provides data for predicting relative in vivo potency among the fluoroquinolones.

Key Words: Bacterial flora—Conjunctiva—Drug resistance—Fluoroquinolone—Ocular infection.

Fluoroquinolones are the newest family of antibacterial agents used in the treatment of ocular infections.[1–5] In Japan, ofloxacin was the first fluoroquinolone introduced for topical ophthalmic use in 1987. Since then, six other fluoroquinolones, norfloxacin, lomefloxacin, levofloxacin, gatifloxacin, tosufloxacin, and moxifloxacin, have been approved for clinical use as eyedrops in Japan. In addition to these compounds, ciprofloxacin has been used clinically in other countries.

Double-masked, randomized clinical trials have shown that single-agent fluoroquinolone therapy using ofloxacin[4] or ciprofloxacin[5] against bacterial keratitis is comparable in efficacy to combining fortified β-lactum agents and aminoglycosides. Their bactericidal activity against the most common gram-positive and gram-negative ocular pathogens is generally excellent, and their high potency has made fluoroquinolones a common choice for the treatment and prevention of ocular infections.

However, as with other antibiotic agents, continued use in a population raises the issue of emerging resistance.[6] Since the introduction of fluoroquinolones for ophthalmic use, the reported incidence of in vitro resistance to fluoroquinolones among bacteria isolated from patients with bacterial keratitis and endophthalmitis has been steadily increasing.[6,7] A previous study by the authors[8] reviewed the database of bacterial flora cultured preoperatively from the conjunctival sac of 1,455 Japanese patients between 1995 and 1999. The incidence of in vitro resistance of bacterial isolates to ofloxacin increased from 13.5% in 1995 to 32.8% in 1999. Although ofloxacin was changed to levofloxacin in 2000, the incidence of resistance to levofloxacin gradually increased from 14.5% in 2000 to 20.5% in 2002.[9]

Some newer fluoroquinolones have been introduced for topical ophthalmic use: gatifloxacin, tosufloxacin, and moxifloxacin. They are sometimes categorized as third-generation fluoroquinolones (i.e., tosufloxacin) and fourth-generation fluoroquinolones (i.e., gatifloxacin and moxifloxacin).[10] Although the clinical benefits of these newer fluoroquinolones have yet to be fully established, their attributes suggest a potential role for the prevention of the increasing incidence of fluoroquinolone resistance among bacterial ocular pathogens. Gatifloxacin and moxifloxacin, especially, which are called 8-methoxyfluoroquinolones, are less likely to engender resistance from single-step topoisomerase mutations. It requires a double mutation in DNA gyrase and topoisomerase IV to establish resistance to 8-methoxyfluoroquinolones.[10] Other potentially beneficial features of 8-methoxyfluoroquinolones are enhanced gram-positive activity relative to older fluoroquinolones and improved drug delivery into the anterior segment of the eye.

This study compared the in vitro effectiveness of bacterial flora cultured preoperatively from the conjunctival sac of patients undergoing intraocular surgery to gatifloxacin, levofloxacin, tosufloxacin, and moxifloxacin.

MATERIALS AND METHODS

Two hundred sixty-six bacterial isolates were collected from the conjunctival sacs of 251 eyes of 224 patients (118 females and 106 males) ranging in age from 6 to 91 years old, who were scheduled

From the Division for Vision Research, National Institute of Sensory Organs, National Tokyo Medical Center, Tokyo, Japan.

Supported in part by a grant from the Ministry of Health, Labor, and Welfare, Tokyo, Japan.

Address correspondence and reprint requests to Dr. M. Yamada, Division for Vision Research, National Institute of Sensory Organs, National Tokyo Medical Center, 2-5-1 Higashigaoka, Meguro-ku, Tokyo 152-8902, Japan; e-mail: yamadamasakazu@kankakuki.go.jp

Accepted June 11, 2007.

DOI: 10.1097/ICL.0b013e31813c102a