Table 1. Reasons for Exclusion From the Present Analysis in the Kumejima Study[a]

Reason for Exclusion	No. of Eyes	
	Right	Left
Screened in own homes	190	190
Difficulties in measuring endothelial cell density[b]	41	40
History of intraocular surgery	604	607
History of acute angle-closure glaucoma attack	1	5
Pseudoexfoliation	25	37
Uveitis	5	5
Corneal disease	6	11
Total	**872**	**895**

[a] These eyes were excluded from the present analysis according to the order shown in the table to avoid repetitive exclusion.

[b] Reasons for the difficulties in measuring endothelial cell density were dementia (3 right and 3 left eyes), bedridden (6 right and 6 left eyes), artificial eye (4 right and 2 left eyes), phthisis (1 right and 4 left eyes), severe corneal opacity (17 right and 12 left eyes), large pterygium (3 right and 3 left eyes), nystagmus or strabismus (4 right and 4 left eyes), and difficulties opening or refusal to open the eye (3 right and 6 left eyes).

including 39 with cornea guttata in both eyes, 53 in right eyes only, and 32 in left eyes only. The prevalence in the right eyes (3.2%) was not significantly different from that in the left eyes (2.5%) ($P=.11$).

The prevalence of cornea guttata adjusted for sex and age is summarized in **Table 2**. The prevalence of cornea guttata was significantly different among subgroups of the 5 age ranges ($P=.003$, Fisher exact test). The grade of cornea guttata was not significantly correlated with age ($P=.32$; $R=0.09$). The prevalence of cornea guttata was significantly different between men and women (2.4% vs 5.8%; $P<.001$, Fisher exact test).

Table 3 shows the results of the comparison between participants with and without cornea guttata. The univariable comparison showed that participants with cornea guttata were older, female, and shorter in stature; had a lower body weight, thinner CCT, and shallower anterior chamber; and smoked fewer pack-years compared with those without (Table 3). Logistic regression analysis with adjustment for only sex (age), only age (sex), or both sex and age (the other factors) showed that greater age, female sex, and thinner CCT were associated with increased risk of cornea guttata (Table 3).

COMMENT

To the best of our knowledge, ours is the first population-based study with a large population size to evaluate the prevalence of cornea guttata in an Asian population. In this study, 124 of 3060 eligible participants (4.1%) had cornea guttata in at least 1 eye. Several studies have reported the prevalence of cornea guttata. A population-based study in Reykjavik reported that the prevalence of cornea guttata was 9.2% in 774 white participants 55 years or older in whom specular microscopy was used for diagnosis.[3] In a non–population-based study conducted in Japan and Singapore with a relatively small number of participants 50 years or older,[4] the incidence of cornea guttata was 6.7% (31 of 465 participants) in Chinese Singaporeans and 3.7% (11 of 299 participants) in Japanese study participants.[4] The prevalence of 4.1% in our study is lower than that in the Reykjavik Eye Study or the study of Chinese Singaporeans. In the study conducted in Japan and Singapore, the authors stated that environmental factors, such as UV light and temperature, may be involved in the occurrence of cornea guttata because there was a difference in the prevalence of cornea guttata in the Japanese and Chinese Singaporeans despite their racial similarity. On the other hand, the prevalence of cornea guttata in the white population reported in the Reykjavik Eye Study was even higher, suggesting that genetic factors contribute to the occurrence of cornea guttata. Moreover, primary central cornea guttata occasionally progresses to Fuchs corneal endothelial dystrophy, which is a major cause of corneal transplantation (9.3%-23.8%) in Western countries[9-13] and only a minor cause (1.7%-3.9%) in Asian countries.[14-16] This finding suggests that genetic or racial factors strongly contribute to the occurrence of Fuchs endothelial dystrophy and cornea guttata. Additional population-based studies in various geographic areas and among various ethnicities are necessary to clarify the effect of environmental and genetic factors on the occurrence of cornea guttata.

In the present study, the prevalence of cornea guttata became higher with increasing age. This finding suggests that primary cornea guttata progresses in an age-dependent manner. In the Reykjavik Eye Study with 774 participants,[3] the mean age of female participants with cornea guttata was significantly higher than that of female participants without cornea guttata, but logistic regression analysis did not show age to be a significant factor associated with cornea guttata. It is possible that the number of participants in our study was large enough to detect the association between the presence of cornea guttata and higher age. On the other hand, the grade of cornea guttata was not significantly correlated with age. Only 3 cases of the highest grade were noted in the group aged 70 to 79 years, whereas none were noted in those 80 years or older. There were 201 participants in the subgroup 80 years or older, whereas there were more than 500 in the other age subgroups. In addition, only 13 persons had cornea guttata in the subgroup 80 years or older. The relatively small number of participants in the oldest subgroup might be responsible for the lack of correlation between the grade of cornea guttata and age. This point needs further investigation.

We found the prevalence of cornea guttata to be significantly different between women and men (5.8% vs 2.4%), and logistic regression analysis with adjustment for age indicated that the prevalence of cornea guttata in women was significantly higher than that in men. The Reykjavik Eye Study reported a higher prevalence of cornea guttata in women (11%) than in men (7%), although the difference was not statistically significant.[3] Similarly, the study conducted in Japan and Singapore reported a higher prevalence of cornea guttata in women than in men (8.5% vs 4.4% in Chinese Singaporeans and 5.5% vs 1.5% in Japanese).[4] Thus, a higher prevalence of cornea guttata in women is likely to be a common find-

Table 2. Sex- and Age-Specific Prevalence of Cornea Guttata in at Least 1 Eye

	Guttata Finding, No. (%) of Participants		Guttata Grade[a]		
	Negative	Positive	1	2	3
Sex					
Male	1513 (97.6)	37 (2.4)	33	3	1
Female	1423 (94.2)	87 (5.8)	74	11	2
Age, y					
40-49	869 (97.2)	25 (2.8)	23	2	0
50-59	767 (96.1)	31 (3.9)	30	1	0
60-69	544 (97.0)	17 (3.0)	12	5	0
70-79	568 (93.7)	38 (6.3)	30	5	3
≥80	188 (93.5)	13 (6.5)	12	1	0
Total	**2936** (95.9)	**124** (4.1)	**107**	**14**	**3**

[a] Guttata grades are explained in the "Examinations" subsection of the "Methods" section.

Table 3. Characteristics of Study Participants With and Without Cornea Guttata and Risk Factor Analysis Using Logistic Regression Analysis Adjusted for Sex and/or Age in the Kumejima Study[a]

Characteristic	Participants With Guttata	Participants Without Guttata	*P* Value[b]	OR (95% CI)	*P* Value[c]
Age, y	62.8 (12.9)	59.1 (12.9)	.002	1.02 (1.01-1.03)	.008
Sex, No. of subjects					
Male	37	1513			
Female	87	1423	<.001	2.38 (1.61-3.53)	<.001
Height, cm	152 (9.3)	155.8 (9.2)	<.001	1.00 (0.97-1.03)	.92
Weight, kg	57.9 (12.1)	61.3 (11.8)	.003	1.00 (0.98-1.02)	.82
BMI	24.9 (3.8)	25.1 (3.6)	.50	0.99 (0.94-1.04)	.74
Diastolic blood pressure, mm Hg	143.4 (23.5)	141.6 (24.0)	.40	1.00 (0.98-1.01)	.53
Systolic blood pressure, mm Hg	77.2 (11.7)	79.1 (13.4)	.08	1.00 (1.00-1.01)	.53
Corneal endothelial density, mm^2	2851.1 (398.3)	2937.2 (392.4)	.05	1.00 (1.00-1.00)	.07
CCT, mm	0.504 (0.038)	0.514 (0.034)	.02	0.001 (0.000-0.892)	.046
Spherical equivalent, D	0.21 (1.82)	0.00 (1.86)	.29	1.00 (0.89-1.12)	.97
Mean keratometric power, D	44.2 (1.5)	44.2 (1.4)	.94	0.92 (0.79-1.08)	.32
Axial length, mm	23.3 (0.8)	23.4 (0.9)	.17	1.03 (0.82-1.31)	.78
Anterior chamber depth, mm	3.00 (0.39)	3.11 (0.39)	.02	0.87 (0.45-1.70)	.69
IOP, mm Hg	14.8 (2.7)	15.2 (3.1)	.18	0.97 (0.90-1.04)	.37
Diabetes mellitus, %	5.6	8.8	.15	0.64 (0.29-1.39)	.26
Hypertension, %	39.5	40.0	.50	0.80 (0.54-1.19)	.27
Smoking, %	24.2	42.8	<.001	0.67 (0.40-1.12)	.13
Contact lens wear, %	1.6	2.4	.44	0.73 (0.17-3.10)	.67
Outdoor work, %	61.3	63.5	.34	1.03 (0.62-1.55)	.88

Abbreviations: BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); CCT, central corneal thickness; CI, confidence interval; D, diopters; IOP, intraocular pressure; OR, odds ratio.

[a] Unless otherwise indicated, data are expressed as mean (SD).

[b] *P* values were the results of comparison between participants with and without cornea guttata by Fisher exact or unpaired *t* test.

[c] *P* values were the results of logistic regression analysis with adjustment for only sex (age), only age (sex), or sex and age (the other factors).

ing among races. This observation is consistent with the fact that Fuchs corneal endothelial dystrophy is observed more frequently in women than in men.[17-19]

In the present study, logistic regression analysis demonstrated that a thinner central cornea is also associated with increased risk of cornea guttata. Higher grades of cornea guttata are likely to be associated with a thicker cornea because cornea guttata occasionally progresses to Fuchs endothelial dystrophy, in which corneal thickness is increased owing to edema. However, in most of the participants with cornea guttata in this study, the grade of cornea guttata was low, and we found no association between cornea guttata and a thicker cornea. On the contrary, cornea guttata was associated with a thinner cornea. This relationship of cornea guttata with CCT requires further investigation.

In the Reykjavik Eye Study, lower weight, lower body mass index, and a history of smoking longer than 20 pack-years were associated with a higher risk of cornea guttata.[3] In the present study, participants with cornea guttata had significantly lower body weight than those without cornea guttata, but this relation was no longer significant after adjusting for age and sex. Thus, in our study, weight and body mass index were not significantly correlated with cornea guttata. Logistic regression analysis in the present study also did not indicate a significant correlation between cornea guttata and smoking.

There are a few limitations in this study. First, we used only a specular microscope to detect cornea guttata because we thought that guttata at an early stage could be detected only with that instrument. However, this could have led to underestimation of the presence of guttata in this population because only a small area in the central cornea can be observed with specular microscopy. Moreover, guttata frequently occur in the paracentral rather than the central area of the endothelium, in which a slitlamp examination is more suitable to detect guttata. In the Reykjavik Eye Study, slitlamp biomicroscopy and specular microscopy were used to detect guttata. Thus, the prevalence of guttata in the Reykjavik study and ours may not be simply compared with each other. Second, the 3762 participants were younger than the 870 nonparticipants. Because the results indicated that the prevalence of cornea guttata became higher with increasing age, it is possible that the prevalence of cornea guttata in the entire population in Kumejima Island is actually somewhat higher than 4.1%.

In conclusion, the prevalence of cornea guttata was 4.1% among individuals 40 years or older in Kumejima, a southwestern island of Japan, by specular microscopic criteria only, which is lower than the prevalence reported in the Reykjavik study. A higher prevalence may have been determined if specular microscopy had been combined with slitlamp biomicroscopy. Older age, female sex, and a thinner cornea were independently associated with a higher risk of cornea guttata.

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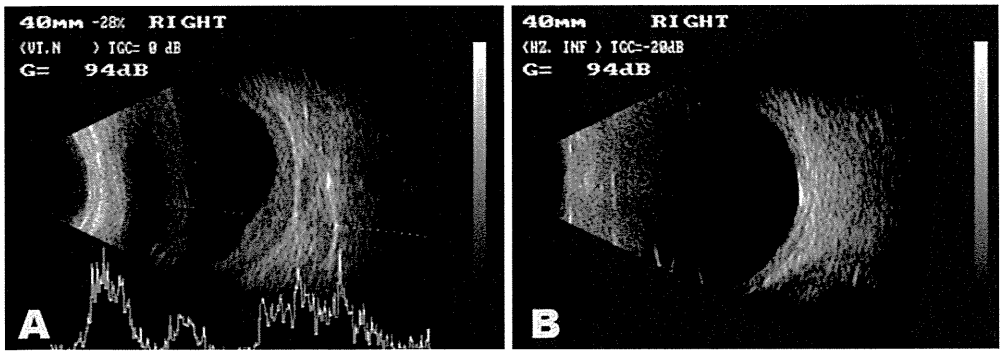

Figure 2A, B. Ultrasonography of the eye. **A** The thickening of episcleral and scleral tissue indicates posterior scleritis. **B** One month after treatment with oral mycophenolate mofetil and subcutaneous methotrexate, the scleral edema resolved.

tapered to oral prednisolone 0.5 mg/kg daily and cyclosporine 1 mg/kg daily because of the development of facial hypertrichosis. Three months later, the patient suddenly developed severe scleral injection (Fig. 1F). B-mode ultrasonography showed posterior scleral thickening (Fig. 2A). Corneal ulceration with de-epithelialization restarted. Immunomodulatory therapy was extended with oral mycophenolate mofetil 500 mg twice daily and subcutaneous methotrexate 20 mg/week. Amniotic membrane transplantation was combined. One month later, the scleral injection and edema resolved (Fig. 2B). However, the corneal ulceration worsened (Fig. 1G) with two episodes of corneal perforation, which were spontaneously sealed after Histoacryl glue and pressure patch application. In view of this refractory course, intravenous administration of infliximab (5 mg/kg) was repeated at 2-week intervals after informed consent was acquired from the patient's parents. After the third infusion of infliximab, the corneal ulceration stopped, and conjunctival hyperemia and ocular discomfort were relieved (Fig. 1H). Because a clear correlation was observed between the erythrocyte segmentation rate (ESR) and levels of C reactive protein (CRP) and the recurrence of keratitis, the dose of systemic immunosuppressant was thereafter carefully adjusted according to the ESR and CRP levels as well as to clinical findings. Systemic immunosuppressants were tapered after 3 months, and remission was maintained during a 12-month follow-up.

Discussion

In the literature published to date, infliximab has been shown to be effective for the treatment of intractable uveitis in children with a very low occurrence of adverse effects.[2–4] Our case demonstrates that infliximab can be also effective in JRA-associated PUK. However, there are reports of increased risk of infection, anaphylaxis, and resistance in the long term in patients receiving this drug.[4,5] Hence, special care should be taken when using infliximab in children.

Keywords: infliximab, juvenile rheumatoid arthritis, keratitis

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Sudden Onset of Amantadine-Induced Reversible Bilateral Corneal Edema in an Elderly Patient: Case Report and Literature Review

Amantadine hydrochloride (Symmetrel) is an achiral polycyclic aliphatic primary amine that is approved for prophylaxis and treatment of influenza A, extrapyramidal signs associated with Parkinson's disease, and drug-induced extrapyramidal reactions. Ocular toxicity is extremely rare,[1–5] and most ophthalmologists likely have little opportunity to see amantadine-induced corneal toxicity. Here we report on the acute onset of amantadine-induced corneal edema and rapid resolution after discontinuation of amantadine therapy in an elderly woman. We also review published reports of similar amantadine-related corneal edema to define the clinical features.

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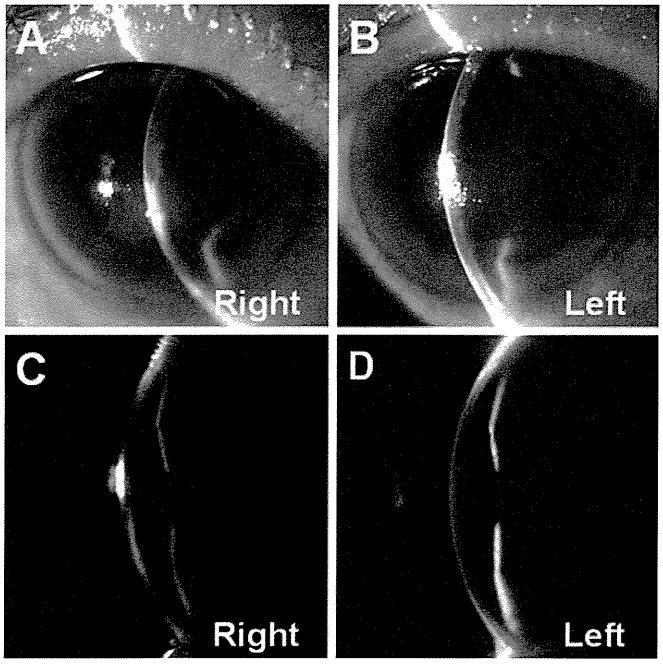

Figure 1A–D. Anterior segment of a 77-year-old woman at her first visit (**A, B**) and at 2 weeks after stopping amantadine (**C, D**). Slit-lamp biomicroscopy revealed bilateral stromal edema and folds in Descemet's membrane, with an intact epithelium at the first visit (**A, B**). Corneal edema had resolved 2 weeks after discontinuation of amantadine (**C, D**).

Case Report

A 77-year-old woman with bilateral corneal edema was referred to our hospital. She reported the sudden onset of bilateral, painless loss of vision, and auditory and visual hallucinations 10 days earlier. Her local ophthalmologist reported that her best-corrected visual acuity (BCVA) was 0.8 bilaterally with normal anterior and posterior segment findings at her last routine eye examination, 3 months before the current presentation, while BCVA had deteriorated to 0.02 OD and 0.01 OS before referral to our hospital. At the first visit, BCVA was 0.05 OD and 0.07 OS. Slit-lamp examination revealed bilateral corneal stromal edema with folds in Descemet's membrane (Fig. 1A, B). The edema was diffuse and the corneal thickness was beyond the range of ultrasound pachymetry with variable readings well above 1000 μm in both eyes. Extraocular movements were normal. Intraocular pressure was 13 mmHg OU. Although the fundus could not be clearly visualized because of corneal opacity, the patient seemed to have an almost normal fundus. Her ocular history was unremarkable, and she had no family history of corneal disease.

Her medical history revealed that she had been started on amantadine therapy by her neurologist. She had complained of numbness in her leg and hands 1 year before the current admission, and her neurologist had started her on amantadine (50 mg three times daily) for tremor 25 days before the current admission. Bilateral loss of vision had occurred 10 days before the current admission. The patient was diagnosed as having amantadine-induced corneal edema. After consultation with the patient's neurologist, amantadine therapy was permanently discontinued. The patient was treated with topical 0.1% betamethasone sodium phosphate (Rinderon A, Shionogi, Osaka, Japan) and sodium chloride eye drops (NaCl 0.5% solution) three times daily to both eyes. Although mild stromal opacity persisted in the inferior cornea of the right eye, her corneal edema resolved 2 weeks after discontinuation of amantadine. Central pachymetry readings were 486 μm in the right eye and 428 μm in the left eye. Visual acuity improved to 0.8 OD and 0.6 OS, but the endothelial cell density was low (901/mm^2 OD and 1134/mm^2 OS). Her auditory and visual hallucinations also resolved when amantadine was stopped.

Comments

Corneal edema in our patient was associated with amantadine therapy and showed rapid resolution after amantadine was discontinued. A summary of case reports from the literature regarding amantadine-induced corneal edema is presented in Table 1. The case described here and the results of previous studies suggest that amantadine-induced corneal toxicity is not dose-dependent and occurs from 2 weeks to 8 years after starting the drug. In addition, prolonged use of amantadine may lead to irreversible corneal edema. Although ocular toxicity is extremely rare, patients receiving amantadine should be monitored for possible changes to their corneal endothelium.

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Keywords: amantadine, corneal edema, corneal endothelium

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Table 1. Summary of case reports of amantadine-induced corneal toxicity

Case no.	Age (years) Sex	Systemic disease	Amantadine dose (duration[a])	BCVA before and after cessation of amantadine		Reversible (recovery time[b]) or Irreversible → Treatment	Steroid Therapy	Reference Author Year (Journal)
				Before	After			
1	64F	Influenza	NA (19 days)	OD20/20 OS 20/20	OD NA OS NA	Reversible (10 days)	None	Blanchard 1990 (Cornea)
2	14M	Neurologic disorder	300 mg/day (NA)	OD 20/400 OS 20/160	OD 20/25 OS 20/25	Reversible (10 days)	None	Hughes 2004 (Cornea)
3	61M	Parkinson's disease	300 mg/day (8 months)	OD 20/70 OS 20/60	OD 20/20 OS 20/16	Reversible (8 days)	None	Kubo 2008 (Parkinsonism Relat Disord)
4	55F	Multiple sclerosis	200 mg/day (6 years)	OD 20/200 OS 20/200	OD NA OS NA	Irreversible → PKP	Topical prednisolone acetate 1%	Jeng 2008 (Ophthalmology)
5	57M	Multiple sclerosis	200 mg/day (2 months)	OD 20/70 OS 20/100	OD 20/25 OS 20/30	Reversible (2 weeks)	Oral prednisone 60 mg/ day	Jeng 2008 (Ophthalmology)
6	44F	Bipolar disorder	200 mg/day (3 months)	OD 20/400 OS 20/400	OD 20/50 OS 20/40	Reversible (1 month)	Topical prednisolone acetate 1%	Jeng 2008 (Ophthalmology)
7	74F	Parkinson's disease	200 mg/day (8 years)	OD 20/200 OS 20/40	OD NA OS NA	Reversible (1 month)	None	Dubow 2008 (Mov Disord)
8	52F	Parkinson's disease	250 mg/day (6.5 years)	OD HM OS HM	OD 20/30 OS 20/60	Reversible (2 weeks)	None	Chang 2008 (Cornea)
9	12F	ADHD	200 mg/day (4 months)	OD 20/200 OS 20/200	OD NA OS NA	NA (NA)	NA	Pond 2009 (Br J Ophthalmol)
10	55F	Parkinson's disease	200 mg/day (Several years)	OD 20/100 OS 5/200	OD NA OS NA	Reversible (NA)	None	Pond 2009 (Br J Ophthalmol)
11	39F	Schizophrenia / tardive dyskinesia	200 mg/day (1 year)	OD 20/400 OS 20/400	OD 6/200 OS 6/200	Irreversible → DSAEK	Topical prednisolone acetate 1%	Koenig 2009 (Eye Contact Lens)
12	39F	Multiple sclerosis	NA (2 months)	OD 20/400 OS 20/400	OD 20/40 OS 20/30	Reversible (2 months)	None	Esquenazi 2009 (Ocul Pharmacol Ther)
13	77F	Tremor	150 mg/day (15 days)	OD 4/400 OS 2/200	OD 20/25 OS 20/32	Reversible (10 days)	Topical betamethasone sodium phosphate 0.1%	Our patient

BCVA, best-corrected visual acuity; NA, data not available; ADHD, Alzheimer disease and attention-deficit/hyperactivity disorder; HM, hand motion; PKP, penetrating keratoplasty; DSAEK, Descemet-stripping automated endothelial keratoplasty.

[a]From the first dose of amantadine to the onset of corneal edema.

[b]From discontinuation of amantadine to recovery from corneal edema.

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Acute *Klebsiella pneumoniae* Interface Keratitis After Deep Anterior Lamellar Keratoplasty

Deep anterior lamellar keratoplasty (DALK) is a relatively new surgical technique that selectively removes the anterior layers of the cornea and preserves the innermost layers, such as the endothelium and Descemet membrane. It has several advantages over penetrating keratoplasty, including avoidance of the risk of endothelial rejection, a closed anterior chamber during surgery, and faster healing.[1]

Infectious keratitis after corneal transplantation is one of the causes of graft failure and is associated with poor visual outcomes. Recently, a new entity after DALK has been described, infectious interface keratitis.[2,3] Ophthalmologists are familiar with interface keratitis after laser-assisted in situ keratomileusis or conventional lamellar keratitis, but to our knowledge, acute infectious interface keratitis after DALK has not yet been reported.

We describe a case of acute infectious interface keratitis after DALK caused by *Klebsiella pneumoniae*.

Case Report

A 35-year-old woman underwent big-bubble DALK for keratoconus in the left eye with no complication during surgery. The patient had no other ocular disease or specific medical history. Postoperative medications included topical chloramphenicol 0.5% and betamethasone 0.1% eye drops four times a day.

On the second day after surgery, the patient complained of ocular pain, red eye, and reduced vision in her operated eye. Her visual acuity was 20/400, and slit-lamp biomicroscopy revealed multiple white deposits at the donor–recipient interface. Over the next hours, the condition progressively deteriorated and the deposits became confluent (Fig. 1). The patient was treated with topical fortified vancomycin (50 mg/ml) and ceftazidime (50 mg/ml) eye drops, each for 30 min. Despite the topical antibiotic therapy, she demonstrated severe stromal involvement and hypopyon, and we encountered a suppurative corneal ulcer.

In the meantime, microbiologic analysis of the donor sclerocorneal rim disclosed donor contamination with *K. pneumoniae*. Because the patient did not respond to the medical regimen, therapeutic penetrating keratoplasty was performed on postoperative day 3 to halt the ulcer's progression and obtain a specimen for bacteriologic and histopathologic evaluations.

First, the sutures were released. Next, the donor cornea was removed and the interface irrigated with vancomycin (50 mg/ml) and ceftazidime (50 mg/ml) solutions for 2 min. Then, the intact Descemet membrane was completely excised and the anterior chamber irrigated with balanced salt solution to wash out the hypopyon. Finally, two peripheral iridectomies were done, and the donor graft was secured with 16 separate 10-0 nylon sutures (Fig. 2A–D). Intravenous antibiotics (vancomycin 1 g/12 h and ceftazidime 1 g/8 h) were started, and the topical fortified antibiotic therapy was continued postoperatively. Fortunately, our patient had an uncomplicated postoperative course. After the surgery, we founded a significant decrease in anterior chamber inflammation, and the graft edema gradually disappeared. From the third day after the operation, betamethasone 0.1% eye drops were started with increasing dosage.

Histopathologic examination of the excised button demonstrated focal areas of inflammation, but no organism. However, the cultures were positive for *K. pneumoniae*. The drug sensitivity profile for this strain was similar to that of the organism isolated from the donor sclerocorneal rim, and both were susceptible to ceftazidime.

Two weeks after the surgery, the corneal graft had become completely clear and the anterior chamber was clear of any cell or flare. Four weeks after the surgery, best-corrected visual acuity had increased to 20/25 with −2 −2.5/160 refraction. At the last postoperative visit, 3 months after the surgery, uncorrected visual acuity was 20/30, best-corrected visual acuity was 20/20, and there was no sign of recurrence (Fig. 2E).

Comments

Incidence of infection following lamellar cornea transplantation is variable; Vajpayee et al.[4] reported an incidence 11.11% greater than that following penetrating keratoplasty, which has been reported to be in the 1.8%–4.9% range.

Up until now, no study has reported the incidence of infectious interface keratitis following DALK. It seems to be a rare complication, and we found just two case reports in a Medline search.[2,3] Kanavi et al.[2] reported two cases of interface keratitis caused by two different *Candida* species after DALK for keratoconus. Fontana et al.[3] reported a case of a 30-year-old man with keratoconus who underwent DALK. All these cases of interface keratitis showed a delayed onset, and *Candida* species were the detected microorganisms.

To the best of our knowledge, this is the first report of *K. pneumoniae* interface keratitis following DALK. Acute onset of clinical signs and symptoms was the major difference between our patient and the patients reported in previous studies. However, similar to those previous cases,

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DSAEKのドナー挿入法

相馬剛至*　西田幸二*

DSAEKにおける内皮グラフト挿入法は大きく3つに分けられる．最初に報告されたのがドナーを2つに折り曲げて前房内に挿入する2つ折り法であるが，内皮損傷が大きいのが問題である．現在，主流になっているのが引き込み法であり，創口の対側のサイドポートから挿入した鑷子を用いて，内皮グラフトを前房内に引き込む．2つ折り法と比較して，内皮損傷が少なく，前房深度が浅い症例でも挿入が容易になったが，挿入時の前房許諾が課題である．近年，開発が進んでいるのがドナー角膜を装填したインジェクターによって挿入するインジェクター法である．今後，より安全で内皮損傷の少ない挿入法の開発が期待される．

はじめに

角膜内皮移植術を大きく2つの手技に分けると，レシピエント角膜の準備とグラフトの挿入に分かれる．レシピエントの準備については，初期の近代内皮移植であるPLK（posterior lamellar keratoplasty）[1]ではホスト角膜実質後部の除去が必要であり，技術的な課題であったが，その後術式の改良が進められ，現在主流であるDSAEK（Descemet's stripping automated endothelial keratoplasty）[2]ではホストのDescemet膜を除去するのみと技術的ハードルが大きく下げられた．一方，ドナー角膜の挿入については，強角膜創もしくは角膜創という狭い創口から接触や伸展により容易に損傷を受ける角膜内皮層を挿入するため，簡便な手技でいかに内皮層へのダメージを少なくするかを課題に開発，改良が進められてきた．

DSAEKに対するグラフト挿入方法は大きく3つに分かれる．最初に報告されたのがドナーを2つに折り曲げて前房内に挿入する2つ折り法．次に，創口の対側のサイドポートから挿入した鑷子を用いて，内皮グラフトを前房内に引き込む「引き込み法」．3つめが，ドナー角膜を装填したインジェクターによって挿入するインジェクター法である．本稿では，これらの3方法を中心に，DSAEKのドナー挿入法の現状と今後の展望について説明する．

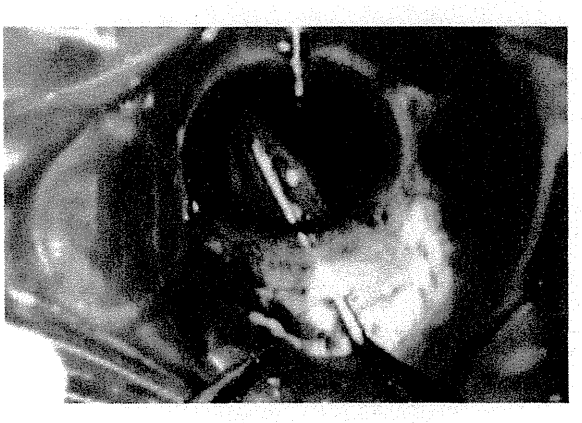

図1　2つ折り法

I　2つ折り法

ドナー角膜の内皮面に粘弾性物質をのせ，内皮面を内側に2つ折りにし（"taco fold"），中央を鑷子で把持した状態で，foldable眼内レンズ挿入の要領で創口から挿入する方法である（図1）．2つ折りと把持による物理的内皮損傷が大きく[3]，加えてアジア人などの前房深度が浅い症例では挿入が困難な場合が多く，挿入できた場合でも内皮層へのダメージが大きな問題となる．

II　引き込み法

現在，主流となっている挿入法である．創口の対側のサイドポートから挿入した鑷子を用いて，内皮グラフト

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■いずれの挿入法においても，グラフトが完全に挿入されていない状態で前房灌流を再開すると，創口よりグラフトが脱出するので注意が必要である．

■引き込み法では，挿入時の前房虚脱がグラフトの内皮損傷，挿入不全の原因となる．

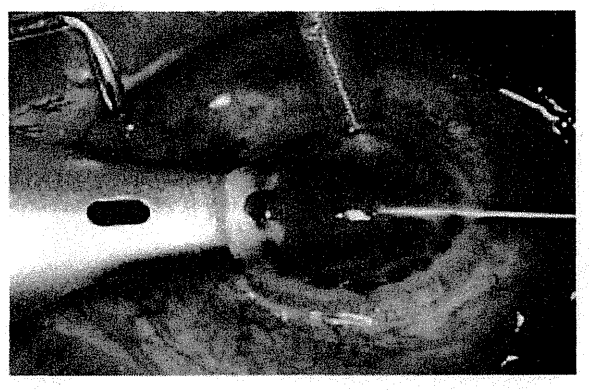

図2　引き込み法

を前房内に引き込む方法である（**図2**）．引き込み法専用の器具（Busin グライド，モリア社）が開発されている（**図3**）．以下，Busin グライドを用いた方法を説明する．Busin グライドに眼内灌流液を数滴滴下して滑りを良くした後に，作製した角膜内皮ドナーをグライドの体部にのせ，内皮保護の目的で内皮面を高粘弾性物質（ビスコート®）で覆う．Busin グライド開口部から挿入した引き込み鑷子でドナーのエッジをつまみ，グライド先端部にまで位置をずらして，内皮ドナーの装填を完成させる．このとき，グラフトがわずかにグライド先端より突出していることを確認する．次に顕微鏡を術野に移動させ，引き込み鑷子を鼻側角膜ポートより挿入して，反転した Busin グライド（ドナー内皮面は下向き）からわずかに突出するドナー内皮の端をつまんで，Busin グライドと平行移動させながら，前房内に Busin グライド先端部を挿入する．この際，前房灌流をいったん off にする．その後ドナー内皮をゆっくりと前房内に引き込みながら挿入する．なお，Busin グライドの単独使用では角膜創からの虹彩および硝子体脱出のリスクがあるため，眼内レンズグライド（アルコン社）を併用するダブルグライドテクニックが報告されている[1]．また，このほか Tan エンドグライド（Network Medical 社）などが引き込み法専用器具として開発されている（**図4**）．

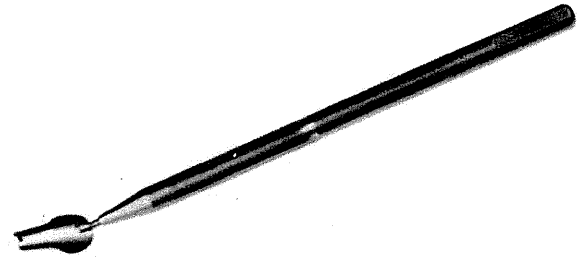

図3　Busin グライド

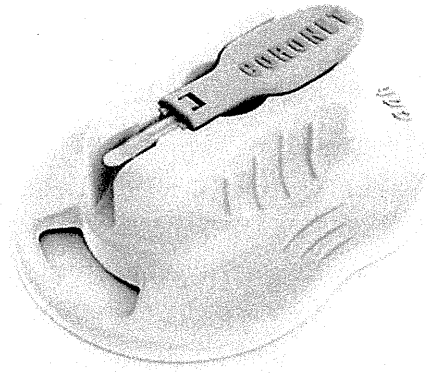

図4　Tan エンドグライド

本法に伴う内皮損傷については，人工前房への挿入実験の結果，2つ折り法では平均32%であったのが，Busin グライドによる引き込み法では平均9%であり，大幅に内皮層へのダメージが軽減されると報告されている[3]．

III　インジェクター法

内皮グラフトをインジェクター内に装填して，インジェクター先端を前房内に設置しグラフトを挿入する方法である．現在，種々のインジェクターが相次いで開発されているが（**図5〜7**），前房スペースで内皮グラフトの保持シートを開く操作が必要であったり，保持シートから内皮グラフトが離れ難いなどまだまだ課題が多いのが現状である．内皮障害が少なくより簡便，安全な器具の開発が望まれる．

■インジェクターは，グラフト挿入時の安定した前房保持に有利なデバイスである．

■グラフトを押し出すのみで挿入できるインジェクターであれば，グラフトへの機械的ストレスは軽減されるであろう．

図5　Neusidl Corneal Inserter（Fischer Surgical 社）

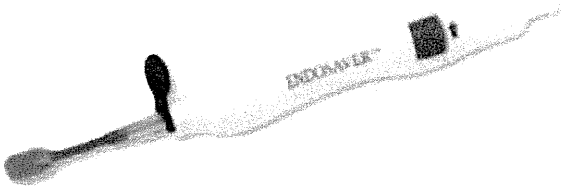

図6　Neusidl Corneal Inserter（Fischer Surgical 社）

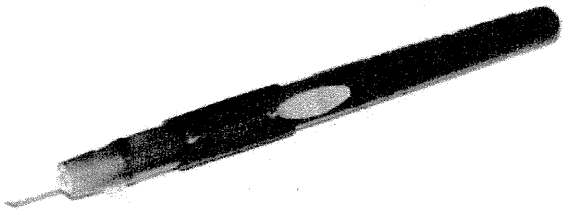

図7　Endoinjecter（KeraMed 社）

おわりに

DSAEK は，PKP（penetrating keratoplasty）と比較して合併症が少なく，乱視が少ないことで良好な視力が得られるため，水疱性角膜症に対する治療法の第一選択となりつつある術式である．DSAEK の克服すべき課題は早期からの内皮細胞減少であるが，これはグラフト挿入時の内皮損傷によるところが大きい．今後，より安全かつ簡便で内皮へのダメージが少ない挿入法が開発されることが，さらなる DSAEK の普及に重要であると考える．

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特　集
角膜移植(眼球提供から移植まで)

3. ドナー検査と保存法

— Examination and preservation of donor cornea —

相馬剛至* 西田幸二*

はじめに

角膜移植の手術成績に影響を与える重要な因子のひとつが，ドナー角膜のクオリティである。ドナー角膜のクオリティと言った場合，角膜としての組織学的な質と生物学的な安全性の両者を含む。角膜の組織学的評価は，細隙灯顕微鏡ならびにスペキュラーマイクロスコープを用いて行う。一方，安全性については提供角膜の微生物汚染を検討するために，提供者自身と提供眼球の両者について微生物学的検査を行い評価する。加えて，ドナー角膜の質の向上と微生物学的検査に必要な時間の確保には，適切なドナー角膜の作製と保存が必須であり，以前より開発が進められてきた。本稿ではこれらのドナー検査とその作製法，保存法について解説する。

I. ドナー(提供者)の血清学的検査

❶ 採　血

眼球摘出前に採血を行い，ドナー自身の血清学的検査を行う。採血の方法を以下に述べる。採血は鎖骨下動静脈にて行う。20ゲージカテラン針を10 cc シリンジに接続し，左鎖骨下の鎖骨内側1/3の部位に刺入する。陰圧をかけながら逆血を確認できる位置まで針を進める。1度で採血できない場合は，方向を変えながら2度，3度針を進めるとうまくいく。感染症検査には，最低1 cc の血清が必要であるので，念のため4～5 cc 採取するようにする。採取した血液は血清分離剤入りの試験管に入れて，静置しておく。

❷ 感染症検査

移植に用いられる角膜・強膜組織は，「角膜移植における提供者(ドナー)適応基準」平成12年1月7日(健医発第25号 厚生労働省健康医療局長発)改正平成22年1月17日(健発0114第2号 厚生労働省健康局長発)の眼球提供者(ドナー)適応基準に基づく(**表1**)。現在，行われている血清学的検査はAIDS(HIV-1，2抗体)，B型肝炎(HBs抗原)，C型肝炎(HCV抗体)，成人T細胞白血病/リンパ腫(HTLV-1抗体)，梅毒である。B型肝炎は角膜移植によってドナーからレシピエントへの感染が報告されている。一方，C型肝炎，成人T細胞白血病/リンパ腫については感染の報告はない。AIDS(後天性免疫不全症候群)については腎移植後にレシピエントにおいて抗体陽性化が報告されて

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Key words：ドナー角膜，組織保存，術前検査，donor cornea, tissue preservation, preoperative examination

表1　眼球提供者(ドナー)適応基準

眼球提供者(ドナー)適応基準

平成12年1月7日(健医発第25号 厚生省健康医療局長発)
改正平成15年11月12日(健発第1112001号 厚労省健康局長発)
改正平成22年1月17日(健発0114第2号 厚労省健康局長発)

1．眼球提供者(ドナー)となることができる者は，次の疾患又は状態を伴わないこと。
(1)原因不明の死
(2)全身性の活動性感染症
(3)HIV抗体，HTLV-1抗体，HBs抗原，HCV抗体などが陽性
(4)クロイツフェルト・ヤコブ病及びその疑い，亜急性硬化性全脳炎，進行性多巣性白質脳症などの遅発性ウイルス感染症，活動性ウイルス脳炎，原因不明の脳炎，進行性脳症，ライ(Reye)症候群，原因不明の中枢神経系疾患
(5)眼内悪性腫瘍，白血病，ホジキン病，非ホジキンリンパ腫等の悪性リンパ腫

2．次の疾患又は状態を伴う提供者(ドナー)からの眼球の提供があった場合には，移植を行う医師に当該情報を提供すること。
(1)アルツハイマー病
(2)屈折矯正手術既往眼
(3)内眼手術既往眼
(4)虹彩炎等の内因性眼疾患
(5)梅毒反応陽性

付記1　2の(1)のアルツハイマー病については，クロイツフェルト・ヤコブ病と症状が類似していることから，鑑別診断を慎重に行う。
付記2　2の(4)の梅毒反応陽性については，提供者(ドナー)が当該状態であっても，提供された眼球より強角膜移植片が作成された場合であって，かつ，当該移植片が3日以上4℃で保存されたものであるときは，感染力がないことに留意すること。また，その場合は，当該移植片につき当該方法で保存したものである旨を併せて移植を行う医師に情報提供すること。
付記3　全層角膜移植に用いる場合は，角膜内皮細胞数が2000個/mm^{2}以上であることが望ましい。
付記4　上記の基準は，適宜見直されること。

いるが，角膜移植ではない。梅毒については，適応基準にあるように強角膜片作成後に3日以上，4℃で保存されたものは，感染力がないと考えられている。このようにB型肝炎以外の病原体では，角膜移植による感染は確認されていないものの，通常の手術時における術前検査基準に準じて血清学的検査を行っている。

その他，Creutzfeldt-Jacob病については，角膜移植による感染例が報告されているが，現時点で有効な検査法がないため，検査対象にはなっていない。また，敗血症などの全身性感染症や眼内悪性腫瘍などは適応基準により除外される。

血清学的検査は，移植可能であるかどうかのスクリーニング目的で行うものであり，迅速に判定結果が得られる定性的検査を施行する。日本アイバンク協会は株式会社SRLと協議のうえ，**表2**に示す血清学的検査をセットで実施するよう依頼している。各アイバンクから分離した血清をSRLがセンターに回収し，一括して検査を行っている。当日の夕方に回収されれば，翌日(土日，休祝日を除く)の正午ごろに結果が判明する。なお，作製した強角膜片についても，使用に際して微生物学的検査を行うのが望ましい。ドナー作製時におけるドナー表面の擦過物もしくは余剰結膜を微生物学的検査に提出し，感染の有無を確認する。加えて，移植終了時に残存ドナー組織や保存液を採取し，細菌検査に

表2　血清学的検査

疾患	検査法
AIDS(HIV-1，2抗体)	PA法
B型肝炎(HBs抗原)	CLEIA法
C型肝炎(HCV抗体)	PA法
成人T細胞白血病/リンパ腫(HTLV-1抗体)	PA法
梅毒	RPR法，TPHA法

供することも勧められる。

II. 強角膜片の作製，検査

ドナー角膜の保存法は全眼球保存と強角膜片保存に分かれるが，現在普及しているのは強角膜片保存である。以前行われていた全眼球保存では，経時的な房水の性状の変化が内皮細胞に影響を与えるため，内皮を健常に維持し得るのはせいぜい2日である。患者の選択や手術準備に要する時間，また微生物学的検査に要する期間を考慮に入れると現実的な保存方法ではない。現在では，房水の死後変化の影響を無視できる強角膜片保存が一般的である。本稿では，強角膜片保存につき述べる。

❶ 細隙灯顕微鏡による観察

摘出した眼球から強角膜片を作製する際に，手持ち細隙灯顕微鏡を用いて前眼部の状態を観察する。日常診療と同様に角膜では上皮びらんや実質混濁(老人環，瘢痕，浮腫など)，血管侵入，デスメ膜皺襞，滴状角膜，角膜後面沈着物などの有無を観察する。角膜実質に混濁がある場合，角膜中央のclear zoneを計測しておく。加えて，虹彩切除や切開の有無，虹彩後癒着，また水晶体の状態(白内障手術既往)を観察する。観察した検眼鏡所見を記録し，内皮スペキュラーの結果から移植の可否および適応(全層もしくは表層移植)について決定する。

❷ 強角膜片の作製

以下の手順に沿って強角膜片を作製する。操作はすべてクリーンベンチ内にて行う。

①クリーンベンチに滅菌した有鉤鑷子(2本)，ディスポメス，眼科用剪刀，キャリパー，抗菌薬点眼，保存液(Optisol™-GS)，滅菌シャーレを準備する。

②摘出された全眼球をシャーレ上に静置し，前項の手順に沿って細隙灯顕微鏡にて観察する。角膜が乾燥しないよう適宜抗菌薬を点眼する。

③移植時の人工前房への設置を考慮し，強角膜片の直径が15 mm以上になるようキャリパーでマーキングし，メスにて強膜切開を行い，作製した切開創から全周性に強膜全層切開を行う。

④切離された強角膜片から虹彩脈絡膜をゆっくり剝離する。この際，粗雑に操作を行うと角膜内皮細胞が剝離，損傷する危険があるため，慎重に行う。眼内レンズ挿入眼では癒着している場合があり，剝離の際に注意を払う必要がある。

⑤強角膜片を保存液中に入れ4℃で保存する。内皮スペキュラーによる観察のため，強角膜片の内皮面を保存液瓶の底面側になるように静置する(**図1，2**)。

❸ 強角膜片の内皮細胞検査

スペキュラーマイクロスコピーを用いた内皮細胞数測定は，ドナー角膜の組織学的な質を評価するうえで必須の検査である。強角膜片における角膜内皮細胞検査には，臨床用のスペキュラーと異なる専用のスペキュラーマイクロスコープが必要である。観察用チャンバーとしてはViewing Chamber®(Chiron社)がある。保存液瓶に専用のアダプターを設置して観察する

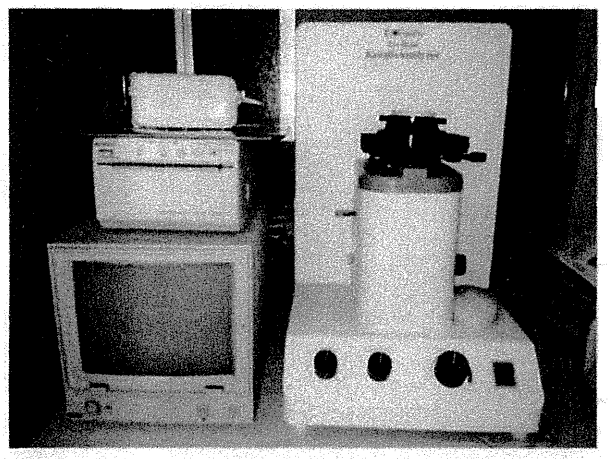

図1　ドナー角膜用スペキュラーマイクロスコピー

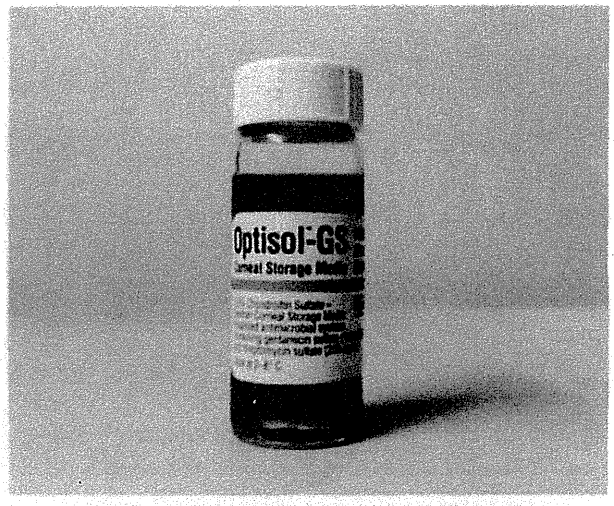

図2　ドナー角膜を保存した保存液瓶

ことも可能である。

観察を行う際には，4℃では観察が困難なため，あらかじめ強角膜片を20～30分室温に静置した後に観察する。内皮細胞が観察できる部位は限られているため，観察可能な場所を探す必要がある。内皮細胞が観察できる場所で細胞を40～50個プロットすることにより，自動的に内皮密度が計算される。異なる3～4カ所で観察を行い，内皮密度数を測定する。

III．ドナー角膜の保存

前述のように，現在では強角膜保存法が主流である。本法には4℃での強角膜保存とさらに保存期間が長い室温～34℃での強角膜器官培養法があり，前者は本邦や米国，後者はヨーロッパの一部の地域で行われている。器官培養法では施設投資や保存中の角膜厚の増大，感染例の増加などが問題として挙げられている。本稿では4℃での強角膜保存について述べる。

強角膜保存液は，内皮細胞に適した環境を組織培養液により与え，膠質を加えて角膜の膨潤を阻止するという発想で開発されてきた。1974年に，M-K medium®(Chiron)と呼ばれる，組織培養液であるTC-199に5%デキストランを加えた保存液が開発された。その後，TC-199に2.5%コンドロイチン硫酸を加えたK-Sol®やMEMをベースとして1.35%コンドロイチン硫酸と1%デキストランを含むDexSol®が開発された。現在，最も使用されている保存液はOptisol™-GSである。これはK-Sol®とDexSol®の利点を組み合わせた保存液であり，抗生剤としてゲンタマイシンとストレプトマイシンを含む。

強角膜片は，角膜実質中のグリコサミノグリカンの吸水性により，電解質のみの保存液中では膨潤し，透明性を失っていく。これは4℃の保存条件では角膜内皮のポンプ機能が低下することとも関係している。Optisol™-GSに含まれるデキストランやコンドロイチン硫酸などの膠質は高浸透圧を生じさせる高分子化合物であるため，長時間にわたって実質の吸水を制御できる。実際，Optisol™-GSで強角膜片を保存した場合，2週間の保存においても，内皮細胞の健常性および角膜の透明性が保たれている。

おわりに

水疱性角膜症をはじめとする角膜疾患では，角膜移植が唯一の治療方法であるが，本邦にお

ける待機患者数は2,600人以上(2009年度)と，ドナー不足が大きな課題である。このような現状において，限られたドナー角膜の最大限有効な活用と手術成績の向上をはかるため，ドナーの検査法および保存法についてさらなる研究，開発を続けていくことが重要である。

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A significant association of viral loads with corneal endothelial cell damage in cytomegalovirus anterior uveitis

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ABSTRACT

Aim The aim of the study was to investigate the correlation between the clinical manifestation and the cytomegalovirus (CMV) viral load in the aqueous humour of patients with CMV anterior uveitis.

Methods Seven patients with CMV-associated iridocyclitis and four patients with CMV-associated corneal endotheliitis were enrolled. Presence of CMV, but not other human herpes viruses, was confirmed by multiplex polymerase chain reaction (PCR). Viral load was measured using real-time PCR. Clinical manifestations were examined using a slit-lamp microscope and ophthalmoscope, applanation tonometer and specular microscope.

Results All 11 patients had unilateral recurrent anterior uveitis with high intraocular pressure and mutton fat keratic precipitates with pigmentation. Stromal oedema of the cornea was found in CMV-associated endotheliitis, but not in CMV-associated iridocyclitis patients. A significant corneal endothelium cell loss was recorded in all 11 patients with CMV-associated endotheliitis and iridocyclitis patients. High viral loads of CMV were detected in the aqueous humour of all 11 patients. A significant association was found between the corneal endothelial cell loss intensity and CMV viral load in the aqueous humour.

Conclusion There is a significant correlation between the CMV viral load and corneal endothelial cell loss in both CMV-associated iridocyclitis and corneal endotheliitis.

INTRODUCTION

Cytomegalovirus (CMV) is a member of the human herpes virus family and is found in latent infections in the majority of the adult population. In immunocompromised hosts, the virus causes necrotising retinitis,[1] but has been thought not to cause any diseases in immunocompetent hosts. However, a previous study showed local production of anti-CMV antibodies in the aqueous humour of an immunocompetent patient with iridocyclitis with elevated intraocular pressure (IOP).[2] In addition, recent studies using qualitative PCR have demonstrated that genomic CMV DNA is present in the aqueous humour of immunocompetent patients with unilateral iridocyclitis[3–6] as follows. Markomichelakis *et al*[3] reported two cases of iridocyclitis with sectoral iris atrophy in which CMV was detected by PCR, and de Schryver *et al*[4] also reported five similar cases. In the recent report by Chee *et al*,[5] they studied if there was a relationship between the CMV viral load in the aqueous and clinical manifestation of the diseases such as either acute or chronic iridocyclitis, eg Posner–Schlossman syndrome and Fuchs heterochromic iridocyclitis. CMV genomic DNA was also detected in the aqueous humour of immunocompetent patients with another inflammatory condition of the eye, ie corneal endotheliitis, in three previous reports.[7–9] Corneal endotheliitis is an inflammatory condition at the corneal endothelium in which keratic precipitates (KPs) develop together with severe stromal oedema in the cornea, whereas iridocyclitis has cells and flare in the anterior chamber with or without KPs but no stromal oedema in the cornea.

The real-time PCR made it possible to measure the viral load quantitatively. Thus, the use of this assay makes it possible to determine the clinical significance of the viral infection in the pathogenesis of human diseases. Our previous report showed a high CMV genomic DNA load in the aqueous humour in an immunocompetent patient with unilateral iridocyclitis with high IOP.[6] However, the correlation between the viral load in the aqueous humour and the clinical manifestation of the disease (iridocyclitis versus corneal endotheliitis) was not investigated. Therefore, we examined if there was any correlation between the CMV viral load in the aqueous humour and the clinical manifestation of anterior inflammatory diseases associated with CMV. We showed a significant correlation between the CMV viral load in the aqueous humour and the endothelial cell damage of the cornea in patients with iridocyclitis and corneal endotheliitis associated with CMV.

MATERIALS AND METHODS

Subjects

Between 2006 and 2008, 11 patients with CMV-associated inflammation in the anterior segment of the eye, ie seven patients with CMV-associated iridocyclitis and four patients with CMV-associated corneal endotheliitis, were enrolled. These patients were from Tokyo Medical and Dental University Hospital (Tokyo, Japan), Miyata Eye Hospital (Miyakonojo, Miyazaki, Japan) and Kyoto Prefectural University Hospital (Kyoto, Japan). Diagnosis was made based on clinical manifestations and the qualitative detection of the CMV genomic DNA in the aqueous humour by the multiplex PCR. The viral load in the aqueous humour was further measured quantitatively by the real-time PCR.

An aliquot of 0.1 ml of the aqueous humour was aspirated with a 30G needle after disinfection and

processed for PCR. Anti-viral therapy was not given before the PCR assay, but topical corticosteroids were given by local ophthalmologists to treat intense anterior uveitis. The interval between the disease onset and the aqueous humour sampling varied among the patients.

Polymerase chain reaction

The aqueous humour samples were centrifuged at 1000 *g* for 5 min and used for multiplex PCR and real-time PCR.[10 11] Multiplex PCR was designed to qualitatively measure the genomic DNA of eight human herpes viruses: herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2), varicella zoster virus (VZV), Epstein–Barr virus (EBV), CMV, and human herpes virus type 6 (HHV-6), type 7 (HHV-7) and type 8 (HHV-8). DNA was extracted from the aqueous humour samples using a DNA minikit (Qiagen, Valencia, California, USA). Multiplex PCR was performed using LightCycler (Roche, Basle, Switzerland). The primers of the glycoprotein gene sequences for CMV were TACCCCTATCGCGTG TGTTC (forward) and ATAG-GAGGCGCCACGTATTC (reverse). The probes used included 3′-fluorescein isothiocyanate: TCGTCGTAGCTACGCTTACAT and LcRed705-5′: ACACCACTTATCTGCTGGGCAGC. Specific primers for the virus were used in conjunction with Accuprim Taq (Invitrogen, Carlsbad, California, USA). PCR amplification conditions used in the current study have been reported previously.[12]

Real-time PCR was only performed for the HHV, with multiplex PCR used to detect the genomic DNA. Amplitaq Gold, with a Real-Time PCR 7300 system (ABI, Foster City, California, USA), was used to perform the procedure. The forward and reverse primers of immediate early (IE)-1 were CATGAAGGT-CTTTGCCCAGTAC and GGCCAAAGTGTAGGCTACAATAG, respectively. FAM-TGGCCCGTAGGTCATCCACACTAGG-TAMRA was used as the probe. The PCR amplification conditions used in the current study were previously reported by Sugita *et al*.[11] When more than 50 copies per tube (5×10^3/ml) were observed, the value of the sample's viral copy number was considered to be significant.

Clinical evaluation

Clinical manifestations of the eye were determined by a slit-lamp microscopic and ophthalmoscopic examination. Each patient underwent best corrected visual acuity (BCVA) measurement using a Japanese standard decimal visual acuity chart (Landolt ring chart) after treatment. Anterior chamber flare was measured by a laser flare photometer (FC-1000; Kowa Electronics, Nagoya, Japan). A photograph of the central cornea using a specular microscope (NONCON ROBO FA-3509; Konan Medical, Nishinomiya, Japan) was used for evaluation of the corneal endothelial cells. In cases of corneal endotheliitis, intense corneal oedema disturbed the measurements of the corneal endothelium, and we measured corneal endothelial cell counts after the inflammation was reduced by the treatment.

Evaluation of corneal endothelial cell loss

The relationship between the CMV viral load in the aqueous humour and the intensity of the corneal endothelial cell loss was assessed. The corneal endothelial cell loss was determined according to the following formula:

$$\text{Corneal endothelial cell loss}(\%) = 100 - (\text{endothelial cell counts in affected eye})/(\text{endothelial cell counts in the fellow eye})\times 100$$

Statistical analysis

Statistical analysis was performed using the Mann–Whitney U test. Statistical significance was set at $p<0.05$. Linear regression analysis was performed using the Spearman's correlation coefficient by rank test.

RESULTS

Clinical manifestations

Nine men and two women ranging in age from 23 to 71 years (mean age 60.6 years) were enrolled in the study. No abnormalities were found in the systemic investigations and laboratory tests. Serology examinations for human immunodeficiency virus were all negative. None of the patients had any history of eye surgery prior to the onset of uveitis. Clinical findings of the CMV-associated iridocyclitis patients (n=7) and corneal endotheliitis patients (n=4) are shown in table 1. A unilateral mild anterior uveitis with high IOP was noted in all 11 patients. There were no significant differences between the iridocyclitis and corneal endotheliitis groups in the cells and flare values in the anterior chamber, nor were there any differences noted for the elevated levels of IOP, KPs, gonioscopic findings and iris atrophy. Stromal oedema of the cornea was seen in all corneal endotheliitis but not in iridocyclitis patients. While the stromal oedema was diffuse in three out of the four patients, it was localised at upper cornea in one of the corneal endotheliitis patients. Representative cases for iridocyclitis and corneal endotheliitis are shown in figures 1 and 2, respectively. As for the IOP elevation, all 11 eyes required anti-glaucoma medications, with two eyes (cases 1 and 2) requiring trabeculectomy. With regard to the iris atrophy, no sectorial iris atrophy was seen in all 11 eyes, although four eyes (two each in the iridocyclitis and the corneal endotheliitis groups, respectively) presented diffuse iris atrophy.

Systemic valganciclovir therapy (1800 mg/day for longer than 3 weeks) in conjunction with topical corticosteroids and

Table 1 Clinical findings in patients with CMV anterior uveitis

Case	Age (years)	Sex	Eye	Diagnosis	Corneal oedema	KPs	Cells in AC	Flare in AC	IOP (mmHg)	Pigmentation in the AC angle	Iris atrophy
1	66	M	R	Iridocyclitis	-	Mutton-fat	1+	17	38	Depigmentation	None
2	62	M	R	Iridocyclitis	-	Mutton-fat	1+	26	40	PAS and pigment	Diffuse
3	56	M	L	Iridocyclitis	-	Mutton-fat	1+	13	44	Depigmentation	Diffuse
4	53	F	R	Iridocyclitis	-	Mutton-fat	1+	13	36	Depigmentation	None
5	71	M	L	Iridocyclitis	-	Mutton-fat	2+	28	25	PAS	None
6	63	M	R	Iridocyclitis	-	Fine	1+	Nt	50	Depigmentation	None
7	23	M	R	Iridocyclitis	-	Fine	1+	Nt	25	Depigmentation	None
8	71	M	R	Endotheliitis	+ (diffuse)	Mutton-fat	2+	151	37	PAS	None
9	67	M	R	Endotheliitis	+ (diffuse)	Fine	1+	14	25	Depigmentation	Diffuse
10	64	F	L	Endotheliitis	+ (superior)	Fine	1+	21	28	Depigmentation	None
11	71	M	R	Endotheliitis	+ (diffuse)	Mutton-fat	1+	12	43	PAS	Diffuse

Information from 11 patients with CMV anterior uveitis were reviewed. Data collected included intraocular pressure and clinical manifestation of the anterior segments in the affected eye.
AC, anterior chamber; F, female; KP, keratic precipitate; M, male; Nt, not tested; PAS, peripheral anterior synechia.

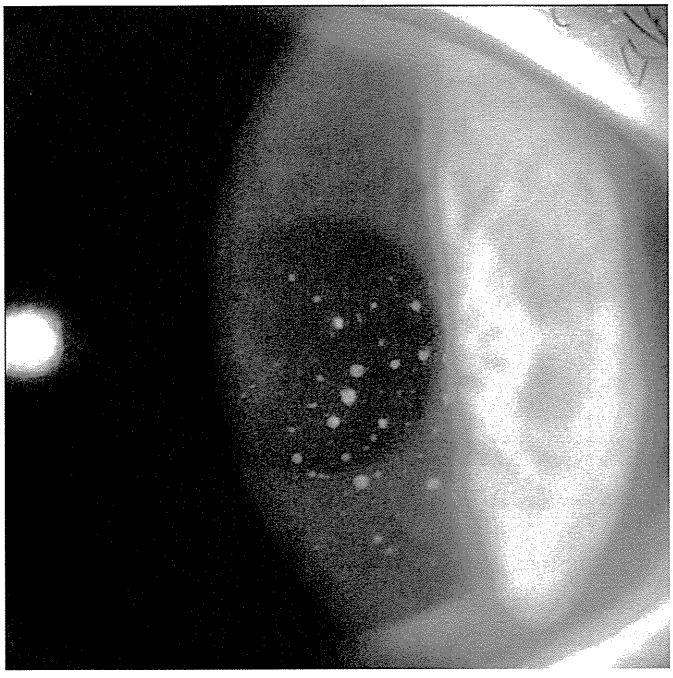

Figure 1 Case 4: Slit-lamp microscopy photo with cytomegalovirus-associated iridocyclitis. Mutton fat keratic precipitates with some pigmentation were scattered within the central area of the cornea. There was mild inflammation found within the anterior chamber.

anti-glaucoma agents effectively controlled the inflammation in the anterior segment of the eye as well as the high IOP.

Corneal endothelial cell loss

Specular microscopic examination revealed significant corneal endothelial cell loss (≥35%) in all 11 patients (table 2). Severe corneal endothelial cell loss larger than 70% was recorded in more than one-half of the endotheliitis group eyes. In contrast, this severe cell loss was observed in one of the seven patients with iridocyclitis.

There were several patients (cases 1, 8, 10 and 11; see table 2) with corneal endothelial cell counts <700 cells/mm^2. Among the patients, three cases had a low visual acuity between 0.3 and 0.6. However, one patient had a good visual acuity of 1.5.

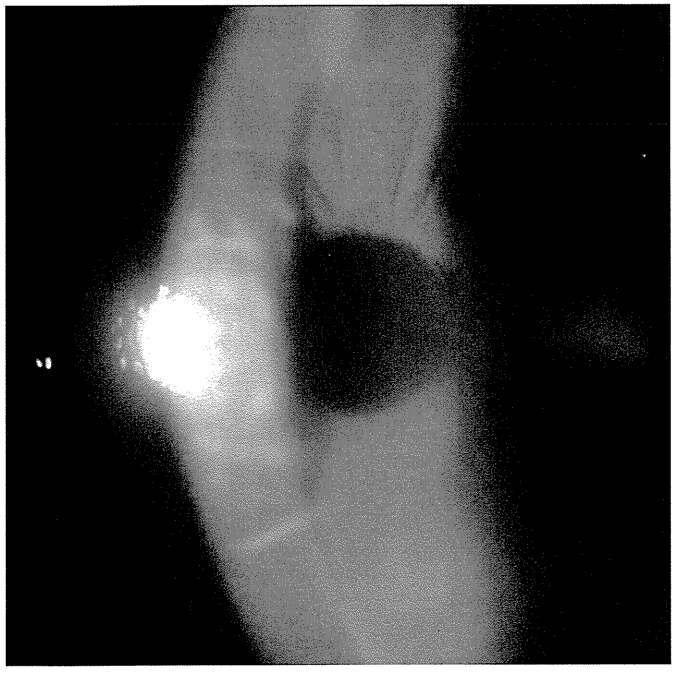

Figure 2 Case 8: Slit-lamp microscopy photo with cytomegalovirus-associated corneal endotheliitis. Diffuse corneal stromal oedema with folds in Descemet's membrane was observed.

PCR analysis of the aqueous humour samples

Multiplex PCR analyses confirmed the presence of CMV genomic DNA, but none of the other human herpes viruses (HSV-1, HSV-2, VZV, EBV, HHV-6, HHV-7 or HHV-8) in all 11 of the patients (table 2).

Quantitative real-time PCR detected significant viral loads of CMV genomic DNA in the aqueous humour of all 11 patients, with values ranging from 5.4×10^3 to 5.9×10^6 copies/ml (table 2). The mean values for the CMV viral load in the iridocyclitis and corneal endotheliitis groups were 9.4×10^5 and 1.2×10^6 copies/ml, respectively. The differences in CMV viral load between the two groups were not significant (p=0.571).

The corneal endothelial cell damage intensity was correlated to the CMV viral load in the aqueous humour. Results of the linear regression analysis demonstrated a positive correlation between the CMV viral load and the corneal endothelial cell loss (Spearman's correlation coefficient by rank test, r=0.664; p=0.036; figure 3).

However, there was no correlation between the interval from the disease onset to the aqueous sampling and the viral load in the aqueous humour (Spearman's correlation coefficient by rank test, r=0.445; p=0.159). Furthermore, the interval from the disease onset to the sampling was not correlated with the corneal endothelial cell damage intensity (Spearman's correlation coefficient by rank test, r=0.373; p=0.239). In addition, there was also no correlation between the viral load and many other ocular findings, such as cells and flare in the anterior chamber, types of KPs, gonioscopic findings, IOP and post-treatment BCVA.

DISCUSSION

The present study analysed ocular manifestations and CMV viral loads in the aqueous humour of patients with CMV-associated iridocyclitis and corneal endotheliitis. Our major findings included: (1) presence of significant corneal endothelial cell loss in both corneal endotheliitis and iridocyclitis tested eyes; and (2) a significant correlation between corneal endothelial cell loss and CMV viral load in the aqueous humour.

Even though it has been demonstrated that viral infections play a significant role in many inflammatory diseases, a qualitative PCR method that is capable of determining the pathological role of these viral infections has yet to be elucidated. If the presence of viral DNA in an affected disease site could be proven, the quantitative determination and correlation with the clinical manifestations of the viral infection could lead to a much deeper understanding of the role of the virus as a pathogenic disease candidate. For example, we have previously reported on two intraocular inflammatory disorders: one involving uveitis associated with human T-cell leukaemia virus type 1 (HTLV-1)[13 14] and the other involving anterior uveitis associated with VZV.[14] In HTLV-1 uveitis, a significantly higher HTLV-1 viral load was detected in the peripheral blood mononuclear cells of the patients compared with asymptomatic HTLV-1 carriers.[13] This viral load was significantly correlated with the vitreous inflammation of the disease.[14] In our report on anterior uveitis associated with VZV, we demonstrated there was a high VZV viral load within the patient's aqueous humour. Furthermore, there was a significant correlation between the viral load and the intensity of the iris atrophy in these patients.[15]

Table 2 Virological analysis and corneal endothelial cell findings in patients with CMV anterior uveitis

Case	Herpes virus DNA		Endothelial cell count (cells/mm^2)		Corneal endothelial cell loss (%)†	Post-treatment BCVA	Interval from onset to sampling (months)
	CMV (copies/ml)	Others*	Affected eye	Fellow eye			
1	2.3×10^5	-	642	2738	77	0.4	96
2	5.5×10^3	-	1633	2869	43	0.8	8
3	1.3×10^4	-	1695	2789	39	1.5	48
4	6.5×10^4	-	1618	3576	55	1.5	24
5	3.5×10^5	-	1445	2608	38	1.2	14
6	5.9×10^6	-	919	2288	45	1.2	16
7	5.4×10^3	-	2512	3917	60	1.2	6
8	1.0×10^6	-	573	2427	76	0.6	12
9	2.8×10^4	-	1427	2262	35	0.7	5
10	1.2×10^4	-	593	2092	72	0.3	4
11	3.6×10^6	-	620	2674	77	1.5	20

Using aqueous humour samples, genomic DNA of the human herpes viruses was measured by qualitative multiplex PCR and quantitative real-time PCR. Corneal endothelial cell count was examined by specular microscopy.
*Herpes viruses excluding CMV, ie herpes simplex virus type 1 and type 2, varicella zoster virus, Epstein–Barr virus, and human herpes virus types 6, 7 and 8.
†Corneal endothelial cell loss was calculated as described in the methods section.
BCVA, best-corrected visual acuity (decimal fraction); CMV, cytomegalovirus.

Although we found that there was a positive correlation between the corneal endothelial cell loss and the CMV viral load in the aqueous humour, there was no correlation between the viral load and many other ocular signs such as cells and flare in the anterior chamber, types of KPs, gonioscopic findings, IOP, post-treatment visual acuity and the interval from the disease onset to the aqueous sampling. These patients had been treated with topical corticosteroids (eg betamethasone) and anti-glaucoma agents (eg timolol and latanoprost) before they were referred to us by local ophthalmologists. These treatments are known to reduce the intensity of anterior uveitis, IOP and other ocular manifestations, but have no effect on recovering the corneal endothelial cell damage, because the corneal endothelial cell damage is barely reversible.

The cells and flare in the anterior chamber were mild in all 11 patients. A possible explanation why the intensity of the inflammatory reaction in the anterior chamber was so mild in this disease might be related to the involvement of the anterior chamber-associated immune deviation (ACAID).[16 17] In an experimental rabbit corneal endotheliitis model, eyes inoculated with inactivated HSV-1 prior to an active HSV-1 infection exhibited less severe inflammatory reactions and corneal endotheliitis. In addition, they also developed an immune deviation to HSV-1.[18] Although CMV-related ACAID has not been previously reported, real-time PCR in the present study demonstrated that CMV genomic DNA was present at high levels within the anterior chamber of the patients. Therefore, it may be that ACAID in response to CMV occurs in the eye, resulting in a relatively mild inflammatory reaction.

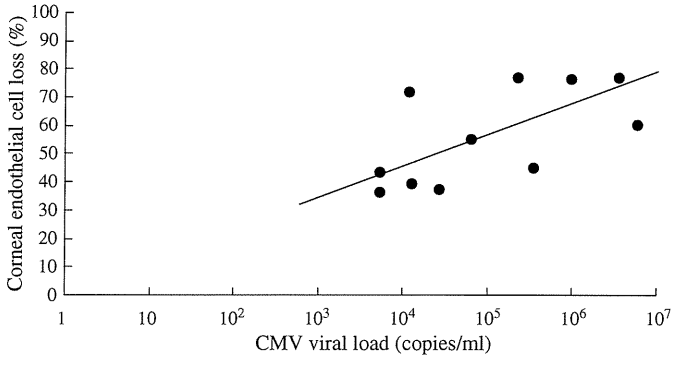

Figure 3 Correlation between cytomegalovirus (CMV) viral load and corneal endothelial cell damage. The CMV viral load was plotted on a logarithmic graph versus the corneal endothelial cell loss (%). The scatter plot shows significant correlation between the CMV viral load and the corneal endothelial cell loss (Spearman's correlation coefficient by rank test, r=0.664; p=0.036).

While our results showed CMV infection in the anterior segment of the eye caused inflammation and corneal endothelial cells loss in immunocompetent hosts, our study cannot answer many other questions. For example, why does CMV cause intraocular inflammation in immunocompetent hosts? Where does the CMV that is detected in the aqueous humour come from? And how is CMV able to cause inflammatory disorder only within the anterior segment of the eye? One possible explanation why our patients developed CMV anterior uveitis is that all our patients had been given topical corticosteroids for a long period of time. This may have contributed to induce local immunosuppressive condition in the anterior segment of the eye and resulted in reactivation of CMV.[8] Further clinical and experimental investigations are necessary to clarify these important questions.

In conclusion, significant corneal endothelial cell damage was detected in all CMV-associated iridocyclitis- and corneal endotheliitis-tested eyes. In addition, a significant correlation was found between corneal endothelial cell loss and the CMV viral load in the aqueous humour.

Competing interests None.

Ethics approval This study was conducted with the approval of the Institutional Ethics Committee of Tokyo Medical and Dental University.

Patient consent Obtained.

Provenance and peer review Not commissioned; externally peer reviewed.

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