# Elevated Aqueous Cytokine Levels in Eyes With Ocular Surface Diseases

NAOHIKO AKETA, TAKEFUMI YAMAGUCHI, TERU ASATO, YUKARI YAGI-YAGUCHI, TERUMASA SUZUKI, KAZUNARI HIGA, TOSHIHIDE KURIHARA, YOSHIYUKI SATAKE, KAZUO TSUBOTA, AND JUN SHIMAZAKI

• PURPOSE: To evaluate cytokine and protein levels in the aqueous humor (AqH) of eyes with ocular surface diseases.

• DESIGN: Prospective consecutive case series.

• METHODS: This study includes 14 patients (aged 62.4 ± 13.7 years) with chronic-phase ocular surface diseases (4 with ocular cicatricial pemphigoid, 5 with chemical burns, 2 with a thermal burn, 2 with Stevens-Johnson syndrome, and 1 with exposure keratitis), 14 matched patients without ocular surface disease (controls with corneal scar), and 30 patients who underwent cataract surgery (healthy controls). AqH samples were collected at the beginning of surgery. AqH levels of cytokines (interleukin [IL]-1α, IL-1β, IL-4, IL-6, IL-8, IL-10, IL-12p70, IL-13, IL-17A, monocyte chemotactic protein [MCP]-1, interferon [IFN]-α, IFN-γ, macrophage inflammatory protein [MIP]-1α, MIP-1β, P-selectin, E-selectin, soluble-intercellular adhesion molecule [s-ICAM]-1, tumor necrosis factor [TNF]-α, granulocyte-macrophage colony-stimulating factor [GM-CSF], IFN-γ-induced protein [IP]-10) were measured using multiplex beads immunoassays.

• RESULTS: The levels of IL-6, IL-10, IL-17A, GM-CSF, E-selectin, P-selectin, and s-ICAM in AqH were significantly elevated in eyes with ocular surface diseases (in pg/mL: 1696 ± 804, 4.0 ± 1.0, 24.3 ± 9.8, 26.0 ± 18.3, 5150 ± 1232, 13122 ± 7219, and 7914 ± 2813, respectively), compared to healthy controls (IL-6: 6.36 ± 0.94, $P = .001$; IL-10: 1.68 ± 0.04, $P = .0006$; IL-17A: 3.7 ± 0.2, $P = .008$; GM-CSF: 2.7 ± 0.3, $P = .007$; E-selectin: 2093 ± 37, $P = .0001$; P-selectin: 3658 ± 137, $P = .0001$; sICAM-1: 1397 ± 119, $P = .008$). The levels of IL-6, IL-17A, E-selectin, and P-selectin in AqH were significantly higher in eyes with ocular surface diseases compared to those with corneal scar (IL-6: 44.1 ± 15.0, $P = .0077$; IL-17A: 4.1 ± 0.7, $P = .034$; E-selectin: 2439 ± 302, $P = .039$; and P-selectin: 5673 ± 1553, $P = .017$).

• CONCLUSIONS: Multiple AqH cytokine levels were elevated in chronic ocular surface diseases. (Am J Ophthalmol 2017;184:42–51. © 2017 Elsevier Inc. All rights reserved.)

IN SEVERE OCULAR SURFACE DISEASES, SUCH AS Stevens-Johnson syndrome (SJS), chemical burns, and ocular cicatricial pemphigoid (OCP), destruction of the corneal epithelial stem cells located at the corneal limbus results in conjunctival invasion, corneal neovascularization (NV), chronic inflammation, and stromal scarring, leading to devastating impairment of vision.[1,2] The critically important functions of the corneal epithelium include the formation of a smooth refractive surface and the role of a barrier against environmental insults.[3] Although these diseases seem to be limited to the ocular surface, a high prevalence of glaucoma and development of cataracts are common comorbidities in severe cases.[4–9] However, little is known about why increased resistance of aqueous outflow or development of cataract occurs in these diseases.

Levels of inflammatory cytokines in the aqueous humor (AqH) have been shown to be elevated during various pathologic processes, such as uveitis,[10–12] post–cataract surgery, and glaucoma.[11–15] We recently showed that elevated cytokines in AqH may predispose the patient to endothelial cell loss,[16] and that iris damage was related to specific cytokine elevations in the AqH.[17] Those findings suggest that a chronic increase in AqH cytokine levels may lead to many pathologic conditions. AqH plays an important role in maintaining a normal homeostatic environment in the eye and is vital for the proper functioning of tissues in the anterior chamber.[18] The immunomodulatory properties of AqH protect the delicate internal structures of the visual axis from the blinding conditions of innate and adaptive immune inflammations.[19–21]

Few studies have been conducted to elucidate the association between chronic ocular surface diseases and intraocular inflammation, yet previous reports showed that tear cytokine levels are elevated in inflammatory ocular surface disease.[22,23] That elevation may affect aqueous cytokine levels, as the breakdown of the corneal epithelial barrier can lead to higher permeability in such ocular surface

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From the Department of Ophthalmology, Tokyo Dental College, Ichikawa General Hospital, Chiba, Japan (N.A., T.Y., T.A., Y.Y.-Y., T.S., K.H., Y.S., J.S.); and Department of Ophthalmology, Keio University School of Medicine, Tokyo, Japan (N.A., T.Y., T.A., Y.Y.-Y., T.K., K.T.).

Inquiries to Takefumi Yamaguchi, Assistant Professor, Department of Ophthalmology, Ichikawa General Hospital, Tokyo Dental College, 5-11-13, Sugano, Ichikawa, Chiba, 272-8513, Japan; e-mail: tym.i.eye.i@gmail.com





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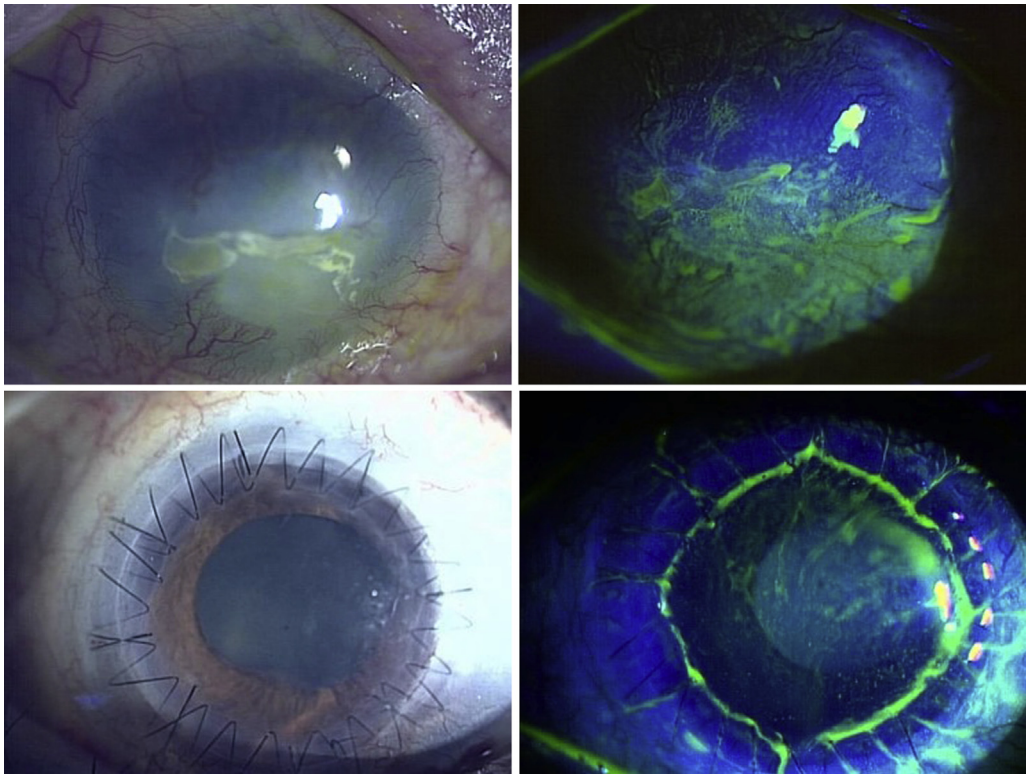

**FIGURE 1. Slit-lamp photographs of a representative case with chemical burn: (Top, left and right) severe neovascularization, stage 2b limbal stem cell deficiency; (Bottom, left and right) 1 month after penetrating keratoplasty.**

diseases.[3] We hypothesized that ocular surface conditions in severe ocular surface diseases may involve intraocular disease pathogenesis via inflammatory cytokine interactions. Hence, in the present study, we examined the association between chronic ocular surface diseases and AqH cytokine levels.

## METHODS

THIS PROSPECTIVE STUDY WAS PERFORMED IN ACCORdance with the Declaration of Helsinki. It was approved by the Institutional Ethics Review Board of Tokyo Dental College, Ichikawa General Hospital (I-15-42). Written informed consent was obtained from all participants.

• **PATIENTS:** Among the 340 consecutive patients who underwent corneal transplantation and/or cataract surgery at Tokyo Dental College from October 5, 2015, to January 31, 2017, 14 patients with ocular surface diseases (Figures 1 and 2; 4 OCP cases, 5 chemical burn cases, 2 thermal burn cases, 2 cases of SJS, and 1 exposure keratitis case), 14 patients with corneal scar (as the control group with corneal disease), and 30 patients with cataracts (as the cataract control group) were included in the present study. Of the 14 patients with ocular surface diseases, 5 underwent cataract surgery, 7 underwent penetrating keratoplasty (PKP), and 2 underwent lamellar keratoplasty (LKP). To test the impact of ocular surface disease on aqueous cytokine levels, we set up a control group of patients with corneal disease fulfilling the following criteria: corneal scar without neovascularization, corneal epithelial defects, and a history of uveitis and ocular surface diseases. We also included 7 patients who had a history of corneal transplantation (PKP in 5 patients and LKP in 2 patients) for the treatment of corneal scar. As a result, 14 patients were included as the control group with corneal scar. The demographics of the participants are shown in Table 1. Because all the eyes with ocular surface diseases exhibited mild-to-severe corneal opacity, we could not accurately assess inflammatory cells or flare in the anterior chamber prior to surgery. However, we performed corneal transplantation or cataract surgery after the treatment for ciliary injection with topical steroids, and we selected the best timing for the surgeries based on the patients' ocular surface conditions.

• **AQUEOUS HUMOR SAMPLES:** AqH was obtained under sterile conditions at the beginning of surgery after retrobulbar anesthesia in corneal transplantation or topical anesthesia in cataract surgery. First, paracentesis was placed at the clear cornea. The AqH samples (70–300 μL) were