Iris Damage Is Associated With Elevated Cytokine Levels in Aqueous Humor

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Purpose. To evaluate the association between iris damage and cytokine levels in the aqueous humor (AqH).

Methods. A total of 201 AqH samples from 201 consecutive patients (mean age 73.7 ± 10.6) were collected at the beginning of corneal transplantation or cataract surgery. Iris damage of each case was assessed from preoperative slit-lamp findings based on its severity. The subjects were classified into three groups: eyes without iris damage (126 eyes), eyes with mild iris damage (51 eyes), and eyes with severe iris damage (24 eyes). The levels of cytokines (IL-1α, IL-1β, IL-4, IL-6, IL-8, IL-10, IL-12p70, IL-13, IL-17a, interferon gamma-induced protein [IP]-10, monocyte chemotactic protein [MCP]-1, IFN-α, IFN-γ, macrophage inflammatory protein [MIP]-1α, MIP-1β, P-selectin, E-selectin, soluble intercellular adhesion molecule [sICAM]-1, TNF-α, and granulocyte-macrophage colony-stimulating factor [GM-CSF]) in AqH were measured by multiplex beads immunoassay.

Results. The levels of aqueous protein, IL-1α, IL-1β, IL-4, IL-6, IL-8, IL-10, IL-17A, MCP-1, TNF-α, E-selectin, P-selectin, and sICAM-1 in eyes with mild and severe iris damage were higher than in those without iris damage (P < 0.033). Multivariate analyses of clinical factors revealed that iris damage was associated with the history of complicated glaucoma, and the number of previous intraocular surgeries. The levels of AqH IL-6, IL-8, IL-13, MIP-1α, TNF-α, and sICAM-1 were significantly elevated in eyes with mild and severe iris damage in phakic eyes, and the levels of AqH IL-8 and sICAM-1 were significantly elevated in eyes with severe iris damage in pseudophakic eyes, compared with the eyes without iris damage (P < 0.045).

Conclusions. Iris damage was associated with the elevation in the levels of aqueous protein and cytokines.

Keywords: iris, aqueous humor, cytokine, immune privilege, glaucoma
Aqueous Cytokine Levels and Iris Damage

Methods

This prospective consecutive study was performed in accordance with the Declaration of Helsinki, and approved by the Institutional Ethics Review Board of Tokyo Dental College, Ichikawa General Hospital (I-15-42). Written informed consent was obtained from all the participants.

Patients

A total of 201 consecutive patients who underwent corneal transplantation and cataract surgery at Tokyo Dental College in the period from October, 2015 to September, 2016 were included. The demographics of the participants are shown in Table 1. The diseases for which surgeries were performed included bullous keratopathy (72 eyes), cataract (61 eyes), corneal scar (28 eyes), Fuchs’ endothelial corneal dystrophy (FECD; 19 eyes), hereditary epithelial/stromal dystrophies (11 eyes); lattice corneal dystrophy, 5 eyes; macular corneal dystrophy, 3 eyes; granular corneal dystrophy, 3 eyes), and keratoconus (10 eyes). We did not perform corneal transplantation or cataract surgery in eyes with active inflammation of the cornea or anterior chamber, and such eyes were not included in the study. We confirmed that the anterior chamber did not contain cells, ciliary injections, or keratoprecipitates using slit-lamp microscopy before surgery. As for flare in the anterior chamber, we measured aqueous protein levels directly, because it was often difficult to assess the flare accurately using slit-lamp microscopy in the eyes with corneal opacity. We excluded 18 eyes with uveal comorbidities (8 eyes with a history of exfoliation syndrome, 3 eyes with chemical burn, 2 eyes with uveal cicatricial pemphigoid, 1 eye with aniridia, 1 eye with endotheliitis, 1 eye with Stevens-Johnson syndrome, 1 eye with exposure keratitis, and 1 eye with iritis), because previous studies showed that aqueous cytokine levels are elevated in eyes with exfoliation syndrome due to breakdown of the blood-aqueous barrier (BAB), and that tear cytokine levels are elevated in inflammatory ocular surface diseases.20,21 which can affect aqueous cytokine levels due to breakdown of the corneal epithelial barrier.22 In the eyes with cataract and bullous keratopathy, when we perform two-step surgery, we always perform cataract surgery first, followed by DSAEK, with an interval of more than 3 months. In the eyes with cataract after penetrating keratoplasty (PKP), we make it a rule to wait for an interval of more than 6 months after PKP to perform the cataract surgery.

AqH Samples

Aqueous humor 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 obtained using a 27-G needle, taking care not to touch the iris, lens, or corneal endothelium. The samples were centrifuged at 3000 g for 5 minutes. The soluble fractions were collected and stored at −80 °C until cytokine levels were measured.

Measurement of Protein Concentration

The concentrations of protein in the AqH samples were determined using the DC protein assay kit (Bio-Rad, Hercules, CA, USA). The reactions were based on the Lowry assay, and were performed according to the manufacturer’s instructions. In brief, BSA in the concentration range of 0.23 to 1.37 mg/mL was used as a standard. Samples (5 μL of BSA and AqH were added to 96-well microplates; this was followed by immediate addition of a mixture containing 25 μL of reagent A+B and 200 μL of reagent C. After 15-minutes incubation at room temperature in the dark, the microplates were read at 690 and 405 nm using a microplate reader (Model550; Bio-Rad). The concentrations were calculated by the subtraction method using a microplate manager system (Bio-Rad).

Measurement of Cytokine Levels

The levels of cytokines (IL-1β, IL-10, IL-4, IL-6, IL-8, IL-10, IL-12p70, IL-13, IL-17A, IFN-α, IFN-γ, monocyte chemotactic protein [MCP]-1, TNF-α, E-selectin, P-selectin, soluble intercellular adhesion molecule [sICAM]-1, granulocyte-macrophage colony-stimulating factor [GM-CSF], macrophage inflammatory protein [MIP]-1α, MIP-1β, and interferon gamma-induced protein [IP]-10) in the AqH samples were measured using Luminex (ProClex kit; Luminex, San Antonio, TX, USA) beads-based multiplex immunoassay according to previous reports.23,24 Briefly, 50 μL of AqH samples were incubated with antibody-coated capture beads in an incubation buffer at room temperature. After 2-hours incubation, the beads were washed thrice using washing buffer and phycoerythrin-labeled streptavidin was added and allowed to bind with in the dark at room temperature for 30 minutes. After three washes with the washing buffer, 150 μL of the reading buffer was added to the plates, and the assays were performed using Luminex 200 system.

Definition of the Severity of Iris Damage

Iris damage was defined as iris depigmentation, laser iridotomy, or iris defect due to intraocular surgeries. The severity grade of iris damage for each case was determined based on its severity from slit-lamp findings. Briefly, healthy eyes are regarded as “no iris damage” (Fig. 1A), eyes with “mild iris damage” are

<table>
<thead>
<tr>
<th>Clinical Factors</th>
<th>Total</th>
<th>No</th>
<th>Mild</th>
<th>Severe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects</td>
<td>201</td>
<td>126</td>
<td>51</td>
<td>24</td>
</tr>
<tr>
<td>Age</td>
<td>75.7 ± 10.6</td>
<td>73.6 ± 10.5</td>
<td>75.3 ± 10.0</td>
<td>70.8 ± 11.7</td>
</tr>
<tr>
<td>Sex (male/female)</td>
<td>80/121</td>
<td>52/74</td>
<td>17/34</td>
<td>11/13</td>
</tr>
</tbody>
</table>

Figure 1. Representative cases with iris damage. (A) No iris damage with a healthy normal iris. (B) Mild iris damage limited to one quadrant. (C) Mild iris damage limited to two quadrants. (D) After laser iridotomy. (E) Severe iris damage in three quadrants. (F) Devastating severe iris damage and atrophy in whole iris.

Table 1. Demographics of the Subjects

<table>
<thead>
<tr>
<th>Iris Damage</th>
<th>No</th>
<th>Mild</th>
<th>Severe</th>
</tr>
</thead>
<tbody>
<tr>
<td>80/121</td>
<td>52/74</td>
<td>17/34</td>
<td>11/13</td>
</tr>
</tbody>
</table>
defined as iris damage limited to only one (Fig. 1B) to two
quadrants (Fig. 1C), or with laser iridotomy (Fig. 1D), and eyes
with “severe iris damage” are defined as iris damage from three
(Fig. 1E) to four quadrants (Fig. 1F). The causes for iris damage
in the samples used in the current study, included laser
iridotomy (LI; 29 eyes), previous corneal transplantation (22
eyes), complicated cataract surgery (14 eyes), complicated
vitreoretinal surgery (5 eyes), glaucoma surgery (trabeculecto-
my in 8 eyes, goniolsynechiolysis in 3 eyes and trabeculotomy in
3 eyes), and trauma (5 eyes).

Data Analysis
To identify clinical parameters associated with iris damage, we
selected the following variables, based on the previous studies
and on our knowledge of iris damage: the presence of
intraocular lens (IOL), a history of corneal transplantation,
complicated glaucoma, diabetes mellitus (DM), axial length,
patient age, and number of previous intraocular surgeries.
Complicated glaucoma was defined as glaucoma with a history
of single or multiple glaucoma surgeries or secondary
glaucoma after complicated intraocular surgeries. Clinical
factors, such as a history of IOL, corneal transplantation,
complicated glaucoma, and DM, were dichotomized for
univariate and multivariate analyses. To assess the association
between the clinical factors and iris damage, univariate
analyses were conducted for each variable using Spearman’s
rank correlations. Multiple linear regression analysis was
conducted using stepwise analysis (Model 1) and backward
elimination analysis (Model 2).

Statistical Analysis
Stata statistical software for Windows, version 14.1 (StataCorp
LP, College Station, TX, USA) was used for all the statistical
analyses, and a P value less than 0.05 was considered
statistically significant. The data are expressed as averages
with SE and median for cytokine levels. The Shapiro-Wilk test
was used to assess whether the data showed a normal
distribution. The Mann-Whitney U-test was used to compare
cytokine levels between each iris damage subgroup.

RESULTS
Aqueous Protein Levels in Eyes With and Without
Iris Damage
The aqueous protein levels in eyes without iris damage were
0.51 ± 0.46 mg/mL, which significantly increased to 0.98 ±
0.14 mg/mL in eyes with mild iris damage (P = 0.0033) and
1.70 ± 0.29 mg/mL in eyes with severe iris damage (P <
0.0001). The aqueous protein level in eyes with severe iris
damage was significantly higher than in eyes with mild iris
damage (Fig. 2, P = 0.016).

Increased Aqueous Cytokine Levels in Eyes With
Iris Damage
The mean cytokine levels in AqH, in terms of iris damage
severity in all the subjects are shown in Table 2. The levels of
IL-1α, IL-1β, IL-4, IL-6, IL-8, IL-10, IL-17A, MCP-1, TNF-α, E-
selectin, P-selectin, and sICAM-1 in eyes with mild and severe
iris damage were higher than those without iris damage (P <
0.035). The levels of IL-13, MIP-1α, IFN-α and IFN-γ in eyes
with mild iris damage were higher than those without iris damage (P
< 0.0056), and IP-10 in eyes with severe iris damage was
higher than that of no iris damage (P = 0.0013). None of the
cytokines in eyes with severe iris damage was lower than that
in eyes without iris damage (P > 0.05), and only IP-10 was
lower in eyes with mild iris damage, compared with that in
eyes without iris damage (P < 0.05). Interferon-γ was higher in
eyes with mild iris damage than in eyes with severe iris
damage. There were no other significant differences in the
cytokine levels between eyes with mild and severe iris damage.

Associations Between Clinical Factors and the
Severity of Iris Damage
Table 3 shows the univariate and multivariate regression
analyses of the association between iris damage severity and
clinical factors. Univariate correlation analysis showed iris
damage severity was correlated with the number of previous
intraocular surgeries (ρ = 0.754, P = 0.0001), presence of IOL
(ρ = 0.516, P = 0.0001), complicated glaucoma (ρ = 0.491, P =
0.0001), history of corneal transplantation (ρ = 0.371, P =
0.0001), and axial length (ρ = −0.170, P = 0.018). Multiple
regression analyses showed that iris damage severity had
significant correlations with the number of previous intraoc-
ular surgeries (β = 0.356, P = 0.0001) and history of
complicated glaucoma (β = 0.355, P = 0.013).

Aqueous Cytokine Levels Stratified With the
Severity of Iris Damage in Phakic and
Pseudophakic Eyes
Having demonstrated that the levels of aqueous protein and
cytokines were increased in eyes with iris damage, we assessed
the levels of aqueous protein and cytokines by dividing the
samples into two categories—phakic and pseudophakic
eyes—because previous report has shown that specific
cytokine levels alter after the cataract surgery.15 In phakic
eyes (Table 4, total 142 eyes), the levels of IL-6, IL-8, IL-13, MIP-
1α, TNF-α, and sICAM-1 in the eyes with mild and severe iris
damage were significantly higher than those in the eyes
without iris damage (P < 0.045). The levels of protein, IL-1β,
IL-17A, GM-CSF, IFN-α, IFN-γ, and P-selectin in the eyes
with mild iris damage were significantly higher than that in the
eyes without iris damage (P < 0.045). The levels of IL-6 and MCP-1
in eyes with severe iris damage were significantly higher than that
in the eyes without iris damage (P = 0.0007 and P = 0.0027,
respectively).

In pseudophakic eyes (Table 5, total 59 eyes), the IL-8 levels
in the eyes with mild and severe iris damage were significantly
higher than that in the eyes without iris damage ($P = 0.0001$ and $P = 0.0031$, respectively). The levels of protein and sICAM-1 in the eyes with severe iris damage were significantly higher than that in the eyes without iris damage ($P = 0.03$ and $P = 0.034$, respectively).

Aqueous Protein/Cytokine Levels and Iris Damage Score (IDS)

Having demonstrated that the aqueous protein level was correlated with the severity of iris damage, we sought to evaluate the association of iris damage and aqueous levels of protein and cytokines in detail. We assessed the aqueous protein and cytokine levels after classifying the iris damage into four grades as reported previously. In brief, IDS 0 indicates no iris damage (Fig. 1A); IDS 1 is defined as iris damage limited only to one quadrant (Fig. 1B); or no iris damage with laser iridotomy (Fig. 1D); IDS 2 is defined as iris damage in two quadrants (Fig. 1C); IDS 3 is defined as iris damage in three quadrants (Fig. 1E); and IDS 4 is defined as iris damage in four quadrants (Fig. 1F). The aqueous protein levels in the eyes with IDS 0 increased from $0.52 \pm 0.05 \, \text{mg/mL}$ (Fig. 3) to $0.86 \pm 0.18 \, \text{mg/mL}$ in IDS1 ($P < 0.001$), $1.22 \pm 0.24 \, \text{mg/mL}$ in IDS2 ($P < 0.001$), $1.62 \pm 0.30 \, \text{mg/mL}$ in IDS3 ($P < 0.001$), and $1.80 \pm 0.52 \, \text{mg/mL}$ in IDS4 ($P < 0.001$). The protein concentration in the AqH significantly correlated with iris damage score ($r = 0.469$, $P < 0.001$). On the contrary, the aqueous cytokine levels were more complex (Table 6; Fig. 4). Although the levels of some aqueous cytokines were significantly elevated in eyes with IDS 1 to 4 compared with those in eyes with IDS 0, there were no statistically significant differences in aqueous cytokine levels among eyes with IDS 1, 2, 3, and 4 (Fig. 4). However, IDS was significantly positively correlated with the levels of IL-1α, IL-1β, IL-2, IL-6, IL-8, IL-10, IL-12p70, IL-13, IL-17A, IFN-γ, MCP-1, TNF-α, E-selectin, P-selectin, sICAM-1, and IP-10 (Table 6; Spearman’s correlation, $P < 0.028$).

**DISCUSSION**

In the current study, we demonstrated that the iris damage was associated with elevated cytokine levels in AqH. Furthermore,
### TABLE 4. Aqueous Cytokine Levels in Phakic Eyes

<table>
<thead>
<tr>
<th>Aqueous Cytokine</th>
<th>No Iris Damage, N = 110</th>
<th>Mild Iris Damage, N = 27</th>
<th>Severe Iris Damage, N = 5</th>
<th>P Value*</th>
<th>P Value†</th>
<th>P Value‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>0.44 ± 0.04 (0.33)</td>
<td>0.82 ± 0.17 (0.61)</td>
<td>0.52 ± 0.11 (0.31)</td>
<td>0.025</td>
<td>0.68</td>
<td>0.43</td>
</tr>
<tr>
<td>IL-1α</td>
<td>59.4 ± 7.7 (45.9)</td>
<td>76.7 ± 13.3 (63.9)</td>
<td>103 ± 23.6 (82.6)</td>
<td>0.083</td>
<td>0.24</td>
<td>0.0007</td>
</tr>
<tr>
<td>IL-1β</td>
<td>2.7 ± 0.6 (1.1)</td>
<td>9.7 ± 4.3 (3.1)</td>
<td>56.5 ± 54.1 (2.5)</td>
<td>0.0003</td>
<td>0.77</td>
<td>0.20</td>
</tr>
<tr>
<td>IL-4</td>
<td>25.4 ± 2.8 (20.3)</td>
<td>42.1 ± 5.8 (27.9)</td>
<td>42.9 ± 14.9 (27.9)</td>
<td>0.0001</td>
<td>0.89</td>
<td>0.07</td>
</tr>
<tr>
<td>IL-6</td>
<td>223 ± 86.2 (80.0)</td>
<td>969 ± 291 (335)</td>
<td>3525 ± 3310 (228)</td>
<td>&lt;0.0001</td>
<td>0.97</td>
<td>0.0024</td>
</tr>
<tr>
<td>IL-8</td>
<td>33.0 ± 8.8 (16.2)</td>
<td>51.1 ± 11.8 (34.8)</td>
<td>496 ± 12.0 (38.8)</td>
<td>0.0001</td>
<td>0.58</td>
<td>0.0031</td>
</tr>
<tr>
<td>IL-10</td>
<td>2.7 ± 0.2 (1.8)</td>
<td>4.5 ± 0.6 (3.3)</td>
<td>4.1 ± 0.7 (5.9)</td>
<td>&lt;0.0001</td>
<td>0.66</td>
<td>0.0019</td>
</tr>
<tr>
<td>IL-12p70</td>
<td>7.6 ± 0.6 (6.4)</td>
<td>12.6 ± 1.7 (8.5)</td>
<td>10.0 ± 1.6 (10.8)</td>
<td>0.0013</td>
<td>0.96</td>
<td>0.046</td>
</tr>
<tr>
<td>IL-13</td>
<td>8.1 ± 0.9 (6.9)</td>
<td>12.1 ± 1.7 (9.5)</td>
<td>14.5 ± 3.8 (13.6)</td>
<td>&lt;0.0001</td>
<td>0.52</td>
<td>0.012</td>
</tr>
<tr>
<td>IL-17A</td>
<td>5.6 ± 0.5 (3.9)</td>
<td>12.9 ± 3.6 (7.4)</td>
<td>9.9 ± 3.8 (7.4)</td>
<td>0.0041</td>
<td>0.99</td>
<td>0.082</td>
</tr>
<tr>
<td>MIP-1α</td>
<td>11.1 ± 1.0 (9.1)</td>
<td>18.0 ± 2.7 (12.8)</td>
<td>16.6 ± 3.9 (13.6)</td>
<td>0.0045</td>
<td>0.83</td>
<td>0.019</td>
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<tr>
<td>MIP-1β</td>
<td>332 ± 47.5 (304)</td>
<td>345 ± 65.9 (279)</td>
<td>424 ± 154 (392)</td>
<td>0.82</td>
<td>0.56</td>
<td>0.50</td>
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<tr>
<td>MCP-1</td>
<td>590 ± 77.3 (458)</td>
<td>634 ± 64.3 (595)</td>
<td>662 ± 72.5 (690)</td>
<td>0.058</td>
<td>0.84</td>
<td>0.0027</td>
</tr>
<tr>
<td>TNF-α</td>
<td>104 ± 8.2 (61.4)</td>
<td>214 ± 54.2 (74.1)</td>
<td>164 ± 26.6 (154)</td>
<td>0.012</td>
<td>0.63</td>
<td>0.0073</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>5.1 ± 11.1 (3.6)</td>
<td>9.9 ± 3.0 (5.1)</td>
<td>13.75</td>
<td>0.036</td>
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<tr>
<td>IFN-α</td>
<td>4.5 ± 0.4 (3.9)</td>
<td>5.8 ± 0.9 (5.0)</td>
<td>4.6 ± 1.1 (6.5)</td>
<td>0.04</td>
<td>0.49</td>
<td>0.86</td>
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<tr>
<td>IFN-γ</td>
<td>62.5 ± 5.7 (52.0)</td>
<td>93.8 ± 12.1 (68.8)</td>
<td>71.6 ± 17.4 (56.8)</td>
<td>&lt;0.0001</td>
<td>0.31</td>
<td>0.27</td>
</tr>
<tr>
<td>E-Selectin</td>
<td>2728 ± 264 (2268)</td>
<td>3424 ± 475 (2677)</td>
<td>2783 ± 777 (2404)</td>
<td>0.043</td>
<td>0.66</td>
<td>0.51</td>
</tr>
<tr>
<td>P-Selectin</td>
<td>5256 ± 472 (3723)</td>
<td>10518 ± 1786 (7540)</td>
<td>6907 ± 1132 (8154)</td>
<td>&lt;0.0001</td>
<td>0.72</td>
<td>0.062</td>
</tr>
<tr>
<td>sICAM-1</td>
<td>1974 ± 248 (1501)</td>
<td>3721 ± 501 (2845)</td>
<td>3147 ± 1000 (2278)</td>
<td>&lt;0.0001</td>
<td>0.68</td>
<td>0.045</td>
</tr>
<tr>
<td>IP-10</td>
<td>406 ± 183 (94.1)</td>
<td>254 ± 82 (129)</td>
<td>422 ± 166 (447)</td>
<td>0.015</td>
<td>0.39</td>
<td>0.16</td>
</tr>
</tbody>
</table>

Mean ± SE (median), protein (mg/mL), cytokines (pg/mL).

* Compared between eyes without iris damage and eyes with mild iris damage, Mann-Whitney U-test.
† Compared between eyes with mild iris damage and eyes with severe iris damage, Mann-Whitney U-test.
‡ Only one sample could be measured using multiplex beads assay among the samples in each group.

Multivariate analysis showed that iris damage was correlated with complicated glaucoma surgery and number of previous intraocular surgeries. These results suggest that iris damage lead to elevation in the levels of aqueous inflammatory cytokines, which would be a useful clinical finding for increased cytokine levels in AqH; however, there was no significant difference in the aqueous cytokine levels between mild and severe iris damage.

Corneal transplants are among the most successful solid tissue transplants. The primary cause of graft failure is endothelial decomposition, even in eyes without any evidence of immunologic rejection. After corneal transplant-
increased in eyes with BK and reduced ECD. We postulate more, we found that the inflammatory cytokine levels in AqH especially in the eyes with severe iris damage (17,35,36) that the presence of complicated glaucoma leads to elevated levels of aqueous cytokines as reported previously.15,39

Furthermore, the study showed that the levels of aqueous protein and cytokine were elevated in the eyes with iris damage both in phakic and pseudophakic conditions. In phakic eyes, the causes of iris damage were LI and surgical iris damage during corneal transplantation and trabeculectomy, whereas the phakic eyes without iris damage included cataract eyes and eyes with FECD. We previously reported the elevation of aqueous cytokines in eyes with a history of LI and past intraocular surgeries.13 To assess the association between iris damage and the cytokine levels in eyes after corneal transplantation, we compared the levels of aqueous cytokines among the different iris damage severity after selecting the eyes after corneal transplantation (Supplementary Table S1, 19 eyes after PKP, 6 eyes after DSAEK, and 2 eyes after anterior lamellar keratoplasty). The intraocular surgeries that caused the iris damage were performed 6 months to 10 years before the AqH collection. Although we have to evaluate the association among the kind of surgeries and the interval between surgeries and AqH collection after increasing the number of subjects, we postulated that iris damage during complicated intraocular surgery might induce the elevated levels of aqueous cytokines over the years in a chronic fashion.

The previous laboratory studies showed that the interaction among iris pigment epithelial cells (I-CB cells in the literature), and the components of the AqH might maintain the homeostasis of the immune system in the anterior chamber. The previous reports on the immunosuppressive effects of the iris pigment epithelium are shown in Table 7.12,40–48 Streilein et al.7 reported that the iris pigment epithelial cells have immunomodulatory properties. The elevated cytokine levels might be attributed to the breakdown of the BAB because the aqueous protein level correlated significantly with the iris damage severity (r = 0.454, P < 0.001). It is tempting to speculate that the results of this study suggest that the lack of immunomodulatory factors from the I-CB cells can change the microenvironment in the AqH, such as elevation in the levels of aqueous inflammatory cytokines, considering the results of previous laboratory reports. In general, cytokines are produced
FIGURE 4. Cytokine levels in aqueous humor stratified based on iris damage score. Levels of some cytokines were higher in the eyes with IDS 1-4 compared with the eyes with IDS 0.
TABLE 7. Literature Review on the Association Between Iris Pigment Epithelium and Anterior Chamber Inflammation

<table>
<thead>
<tr>
<th>Author (y)</th>
<th>Cell Line/Animal/Model/Subjects</th>
<th>Molecules</th>
<th>Principal Source</th>
<th>Principal Cellular Targets and Biologic Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Streilein, et al. (1990)</td>
<td>Cell line MLR</td>
<td>TGF-β</td>
<td>AqH</td>
<td>T cell; suppress proliferation</td>
</tr>
<tr>
<td>Streilein, et al. (1991)</td>
<td>Cell line MLR</td>
<td>NA</td>
<td>I-CB</td>
<td>Immune cells; increased cell infiltration in the iris and aqueous protein levels</td>
</tr>
<tr>
<td>Suzuma, et al. (1997)</td>
<td>Rat EIU</td>
<td>P-selectin</td>
<td>I-CB</td>
<td>Immune cells; increased cell infiltration in the iris and aqueous protein levels</td>
</tr>
<tr>
<td>Suzuma, et al. (1998)</td>
<td>Rat EIU</td>
<td>E-selectin</td>
<td>I-CB, retina</td>
<td></td>
</tr>
<tr>
<td>Marie, et al. (1999)</td>
<td>Rat EIU</td>
<td>TNF-α, IL-6, IL-13</td>
<td>I-CB</td>
<td>Immune cells; IL-13 suppresses cell infiltrates in the iris by downregulating TNF-α and IL-6</td>
</tr>
<tr>
<td>Yoshida, et al. (2000)</td>
<td>Cell line/ mouse MLR/ACAID</td>
<td>TGF-β</td>
<td>I-CB/AqH</td>
<td>T cell; suppress proliferation</td>
</tr>
<tr>
<td>Lemaitre, et al. (2001)</td>
<td>Mouse EIU</td>
<td>IL-13</td>
<td>Exogenous IL-13</td>
<td>Exogenous IL-13 suppresses cell infiltrates in the iris by downregulating MCP-1 and MIP</td>
</tr>
<tr>
<td>Mo, et al. (2003)</td>
<td>Mouse PDS/ACAID</td>
<td>CD95</td>
<td>I-CB</td>
<td>1-CB contribute to ACAAID and suppress T cell proliferation</td>
</tr>
<tr>
<td>Ishii, et al. (2016)</td>
<td>Human EK</td>
<td>NE</td>
<td>NE</td>
<td>Iris damage was associated with endothelial cell loss, leading to graft failure</td>
</tr>
<tr>
<td>The current study</td>
<td>Human Cataracts/corneal disease</td>
<td>Cytokine</td>
<td>NE</td>
<td>Iris damage was associated with elevated cytokine levels in AqH</td>
</tr>
</tbody>
</table>

MLR, mixed lymphocyte reaction; NA, not available; EIU, endotoxin-induced uveitis; ACAID, anterior chamber associated immune deviation; PDS, pigment dispersion syndrome; Treg, regulatory T cell; EK, endothelial keratopathy; NE, not evaluated; BK, bullous keratopathy.

in immune cells in response to specific stimuli, such as infection, trauma, or autoimmune diseases. Hence, further evaluation of the causes of chronic inflammation in the anterior chamber is needed in future studies.

Because cytokines are very sensitive, some systemic condition can affect the aqueous cytokine levels, as reported for the elevation of tear cytokine levels with deterioration of rheumatoid arthritis. Systemic inflammatory conditions can also affect AqH cytokine levels via elevation of serum cytokines or breakdown of the BAB. We thoroughly checked the clinical records of all patients regarding steroid use and systemic diseases. There were nine patients with systemic inflammatory diseases: three patients with rheumatoid arthritis, two patients with asthma, one patient with atop dermatitis, one patient with polymyositis, one patient with polynuertitis, and one patient with Sjogren syndrome (2 patients without iris damage, 1 patient with mild iris damage, and 4 patients with severe iris damage). We compared the aqueous protein and cytokine levels between these nine patients and 192 patients without systemic diseases and found that there were no statistically significant differences in cytokine levels between the eyes with mild and severe iris damage (Supplementary Fig. S1). Iris damage was associated with various clinical factors and the measurement of cytokines in the future. Second, whereas the aqueous proteins level was directly correlated with the severity of iris damage (Figs. 5), there were no statistically significant differences in aqueous cytokine levels between the eyes with mild and severe iris damage (Supplementary Fig. S1). Iris damage was associated with various clinical factors and the measurement of cytokines in the future. Thirdly, we included only Japanese eyes with brown pigmented iris. In Asian eyes, iris damage is easy to identify, compared with in eyes with less pigmentation as in the iris of Caucasian eyes. We need further studies to evaluate the effect of iris damage on the levels of aqueous cytokines in other races. Forth, the interval between the previous glaucoma surgery and AqH collection can affect the aqueous levels of cytokines. The mean interval was 14.4 ± 14.8 years, ranging from 2 to 54 years (total 15 eyes: 12 eyes after trabeculectomy and 3 eyes with multiple glaucoma surgeries). The aqueous levels of protein and cytokines were not significantly correlated with the interval between the glaucoma surgery and AqH collection, although we have to increase the number of subjects after glaucoma surgery. The other limitation is that we could not...
grade flare in the AqH owing to the presence of corneal opacities in some of the subjects.

In conclusion, we have shown that the iris damage is associated with elevated levels of aqueous protein as well as inflammatory cytokines, such as IL-1α, IL-1β, IL-6, IL-8, IL-10, IL-13, IL-17A, MCP-1, TNF-α, E-selectin, P-selectin, MIP-1α and sICAM-1. The multivariate analyses revealed that the iris damage severity is associated with the number of previous intraocular surgeries and with the presence of complicated glaucoma. Thus, the preexisting iris damage can be one of the useful clinical parameters for chronic breakdown of BAB and elevated inflammatory cytokines, although there was no direct correlation between the extent of iris damage and the level of cytokines.

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References


