The Level of Inflammatory Tear Cytokines is Elevated in Congenital Aniridia and Associated with Meibomian Gland Dysfunction

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PURPOSE. To investigate the tear cytokine profile in congenital aniridia, and correlate cytokine levels with ophthalmologic findings.

METHODS. We examined 35 patients with aniridia and 21 healthy controls. Tear fluid was collected with Schirmer I test and capillary tubes from each eye, and the concentration of 27 inflammatory cytokines determined using multiplex bead assay. Eyes of all participants were examined with tests for dry eye disease, including evaluation of meibomian glands (meibography). Differences in cytokine levels between the two groups were analyzed, and correlations between cytokine concentrations and ophthalmologic findings in the aniridia group investigated.

RESULTS. The concentrations of six tear cytokines were significantly higher in aniridia patients than controls in both eyes, and included interleukin 1β (IL-1β), IL-9, IL-17A; cotaxin; basic fibroblast growth factor (bFGF/FGF2); and macrophage inflammatory protein 1α (MIP-1α/CCL3). The ratio between the anti-inflammatory IL-1RA and the proinflammatory IL-1β was significantly lower in patients than controls in both eyes (P = 0.005 right eye and P = 0.001 left eye). Increasing concentration of IL-1β, IL-9, IL-17A, FGF2, and MIP-1α correlated with parameters for meibomian gland dysfunction (MGD) in the aniridia group, including increasing atrophy of meibomian glands, and shorter break-up time of the tear film.

CONCLUSIONS. A number of pro-inflammatory cytokines are significantly elevated in tear fluid from aniridia patients, and correlate with parameters for MGD in aniridia. Increased inflammation of the ocular surface may be a factor in the development of MGD in aniridia patients, and explain the high prevalence of MGD and dry eye disease in these patients.

Keywords: inflammation, cytokine, tear film, aniridia, dry eye

Congenital aniridia is a rare disorder that affects both anterior and posterior parts of the eye, including the cornea, iridocorneal angle, iris, lens, retina, and optic nerve. The prevalence in Norway is 1:76,000.1 Hypoplasia of the iris and of macula lutea is present in most patients and commonly results in photophobia, considerably reduced visual acuity, and nystagmus. Progressive ocular disorders such as glaucoma, cataract, and aniridia-associated keratopathy (AAK) are common.1,2 Mutation in the PAX6 gene is the major genetic explanation for aniridia.3

The prevalence of dry eye disease (DED) in aniridia is high.4 Besides, significant correlation has been found between DED and the severity of AAK.5 As AAK is a sight-threatening complication, investigations of DED in aniridia patients may provide important knowledge that brings better visual outcome.

Increased levels of various inflammatory cytokines in the tear fluid have been demonstrated in DED.6 Moreover, elevated concentration of these cytokines was correlated with increased severity of DED. Inflammation can lead to ocular surface epithelial disease and altered corneal epithelial barrier function in DED.7 DED may thus initiate AAK through activating inflammatory pathways. In turn, AAK might exacerbate DED through inducing inflammatory cascades, creating a vicious

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circle. To our knowledge, this is the first published study to comprehensively analyze inflammatory tear cytokines in aniridia patients. By investigating the tear cytokine profile in these patients, we aim to add clues to the pathogenesis of DED in aniridia, which may pave the way for improved treatment of ocular surface pathology.

**Methods**

**Study Subjects**

The study comprised 35 patients (21 females) with congenital aniridia (age range: 9–72 years) and 21 healthy controls (12 females; age range: 19–65 years). The aniridia group was recruited through the patient organization Aniridia Norway. Patients had previously been diagnosed with aniridia, and the diagnosis was confirmed by ophthalmoscopy before inclusion in the study. Ethics committee approval was obtained from the Norwegian Regional Committees for Medical and Health Research Ethics (Application no. 2014/382) and the study was conducted in accordance with the tenets of the Declaration of Helsinki. All participants gave written consent after receiving oral and written information about the study.

**Dry Eye Examination, Sample Collection and Storage**

Results from dry eye examinations were used for correlation analysis with cytokine concentrations. The results from dry eye examinations were presented in a separate manuscript.

**Measurement of Tear Production and Collection of Tear Fluid**

Tear meniscus height was measured with a corneal topographer (OCULUS Keratograph 5M; OCULUS, Wetzlar, Germany). Tear fluid was collected with Schirmer I tear test using test strips (Schirmer Tear Test Strips; Haag-Streit UK, Essex, UK), and capillary tubes. Collection was performed without topical anesthesia. Wearing gloves, the examiner placed the test strip (Haag-Streit UK) at the lateral eyelid margin. The participants had their eyes closed during the procedure, and both eyes were tested simultaneously. The strip was removed after 5 minutes, and the wetted part measured on a millimeter scale up to the indentation line. The strip was then stored in a cuvette containing 500 mL phosphate buffered saline, which was subsequently placed in a deep freezer at −80°C.

Tear production was further measured with a phenol red thread test (Tianjin JingMing New Technological Development Co., Tianjin, China), without topical anesthesia and for 15 seconds.

Next, tear fluid was collected in capillary tubes. To obtain contact with the tear meniscus, the tube end was placed at the lateral lower eyelid margin, avoiding contact with the eyelid or bulbar conjunctiva. Samples were collected from both eyes, and the capillary tubes stored at −80°C.

**Measurement of Tear Film Quality and Ocular Surface Staining**

Tear film osmolarity was measured with commercial equipment (TearLab; TearLab Corp., San Diego, CA, USA). Tear film lipid layer was evaluated by recording interference images on the keratograph during several blink intervals (lipography). The test was designated as positive if spread of the lipid layer could be observed, or negative if not.

Fluorescein break-up time was measured after placing 5 μL of 2% fluorescein sodium (Minims Fluorescein Sodium 2%; Bausch & Lomb House, Surrey, UK) into the conjunctival sac. The time from a blink to break up of the tear film was then measured. Without delay, punctate fluorescein vital staining of the conjunctiva and cornea was then assessed using the Oxford grading scheme.8

**Corneal Sensitivity**

Central corneal sensitivity was measured using an esthesiometer (Cochet-Bonnet; Luneau Ophthalmology, Chartres, France). The esthesiometer contained a 60-mm long monofilament, which was retracted in 5-mm steps from its full length, until a blink response was elicited.

**Examination of Eyelids**

Eyelid pathology was denoted as either present or absent in each of the following eight groups: debris at the eyelid margin, edema and thickening of the eyelid, irregularity of the lid margin, hyperemia in the eyelid, telangiectasia in the eyelid, occlusion of gland orifices at the lid margin, and presence of conjunctival papillae or conjunctival hyperemia.

Expression of meibomian glands (MGs) was performed in lower eyelid, and expressibility based on number of central five glands that expressed secretion. Quality of expressed secretion was graded according to recommendations from the International Workshop on meibomian gland dysfunction.9

MGs were visualized taking infrared images with the keratograph. MG loss in upper and lower eyelid was evaluated subjectively and staged according to a four-point scale (meiboscore). The percentage area of MG loss was defined as the area of MG loss in relation to the total visible tarsal area and given a score from 0 to 3. A score of 0 represented an area of MG loss of 0% to 25%; a score of 1 represented an area of MG loss of 26% to 50%; a score of 2 represented an area of MG loss of 51% to 75%; and score of 3 represented an area of MG loss of >75%.

**Clinical Evaluation of the Eye**

Anterior part of the eye was examined with a slit-lamp biomicroscope. AAK was graded according to a modification of Mackman’s classification.10 Stage 0 indicated no involvement of the cornea; stage 1 referred to ingrowth of conjunctival tissue in the peripheral cornea from less than 360° of the corneoscleral limbus; stage 2 related to 360° conjunctivization of the peripheral cornea, but clear central cornea; and stage 3 described presentation as in stage 2, but included conjunctival vascularization and/or stromal involvement of the central cornea. Amount of iris hypoplasia was classified as either total, if no iris tissue was visible, or partial if some tissue could be seen. Stage of cataract was graded according to the Lens Opacities Classification System III.11

**Analyses of Tear Fluid**

Cytokine concentration in the tear fluid was measured using immunoassay technology (Bio-Plex XMap; Bio-Rad Laboratories, Inc., Hercules, CA, USA) with a commercial instrument (Luminex IS 100; Luminex Corp., Austin, TX, USA) powered by commercial software (Bio-Plex version 6.0.1; Bio-Rad Laboratories, Inc.). Do modest sample volumes, the multiplex assay was performed with half the standard sample volume, beads, detection antibody, and streptavidin-phycocerythrin. To optimize the assay for low-level detection, screening was carried out with an additional standard point along with increased
incubation time of beads and sample up to an hour. The broad
screening kit (Bio-Plex Pro Human Cytokine 27-plex Assay, Cat.
50500KCAFOY; Bio-Rad Laboratories, Inc.) contained 27
different cytokines: IL-1β, II-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-
10, IL-12p70, IL-13, IL-15, IL-17A, IL-17A, IL-18, IL-1RA (receptor antagonist);
etoxin, basic fibroblast growth factor (bFGF/FGF2); granulo-
cyte colony-stimulating factor; granulocyte macrophage colo-
nystimulating factor; interferon gamma; interferon gamma-
induced protein 10; monocyte chemotactant protein 1; macrophage inflammatory protein 1β (MIP-1α/CCL3); MIP-1β ; platelet-derived growth factor bb; regulated-on-activation
normal T cell expressed and secreted; tumor necrosis factor alpha; and vascular endothelial growth factor.

Not all participants completed the Schirmer and capillary
test, and from some of them no visible tear fluid was collected
in the capillary tubes. To ensure sufficient tear fluid for
analyses, 11 Schirmer tests with 5-mm wetting or less were
discarded (five from aniridia group and six from control
group). All tear samples were thawed on ice and 6 µL of tear
fluid from capillary tubes were mixed with 24 µL of sample
diluent and added to the test plate. In a small number of cases
in which tear fluid volume was less than 6 µL, sample diluent
was added to final volume of 30 µL, and the diluent factor
adjusted accordingly. The cuvettes with Schirmer strips
stored in phosphate buffered saline were vortexed, and 25
µL of the suspension was added directly to the plate. Indivi-
dual sets of samples from patients were run on the
same plate. Plates were washed with a magnetic plate washer
(Bio-Plex Pro Wash Station; Bio-Rad Laboratories, Inc.). The
standard curve for each cytokine was performed in duplicate
using recombinant proteins in a 4-fold dilution. Results were
validated by a five-parametric logistic curve modeling and
used to determine the protein levels in the tear fluid samples.
Longitudinal controls were applied to obtain inter- and
intracontrol coefficient of variation. All values were in
acceptable range according to recommendations from the
manufacturer (intraperpertinent coefficient of variation <11 and
intracontrol coefficient of variation >21). Protein concentra-
tions in the Schirmer strip suspensions were adjusted as a
direct function of the total length of wetted strip, which was
calculated from the Schirmer test result.

Quantitative and Statistical Analyses
Statistical analyses were performed with statistical software
(SPSS software version 23.0; IBM Corp., Armonk, NY, USA).
Cytokine levels were calculated in four groups: Schirmer test
right eye (SOD) and left eye (SOS), and capillary test right eye
(COD) and left eye (COS). Reported results are presented as
means ± standard deviation. Normal distribution was analyzed
using Shapiro-Wilk test and histogram analysis, and Mann-
Whitney U test applied to calculate differences in cytokine
levels between aniridia and control subjects. The alpha level
was set to 0.05 and significant results determined by the Holm-
Bonferroni method to correct for multiple comparisons.
Correlation analyses between cytokine levels and clinical
parameters in the aniridia group were performed using
Spearman’s rank correlation and values of P ≤ 0.05 considered
as significant.

RESULTS
Mean age in the aniridia group was 34.9 ± 18.7 years (range: 9–72
years) and 31.2 ± 13.9 years (range: 19–65) years in the control
group. Table 1 shows number of tests and mean age of
participants in the four subgroups combining Schirmer/
capillary test and right/left eye. In aniridia patients, mean
Schirmer test result was 27.5 ± 9.4 mm in right eye and 27.7 ±
9.1 mm in left eye.

Comparison of Cytokine Levels Between Aniridia
and Control Groups
In the SOD group, the concentrations of five cytokines were
significantly higher in aniridia patients than control subjects; in
the COD group, the levels of six cytokines were significantly
elevated in aniridia group (Table 2); in the SOS group, 17
cytokines had increased concentration in aniridia subjects; and
in the COS group, the levels of two cytokines were higher in
aniridia than controls (Table 3). None of the cytokines tested
had significantly lower concentrations in the aniridia group
than in the control group.

The concentrations were significantly higher for seven
cytokines in the right eye and 17 in the left eye in either one or
both test modalities. Consequently, a lower number of tear
cytokines had increased concentrations in the right versus
the left eye, but all the cytokines with elevated concentrations in
right eye were also raised in left eye, except for IL-2. Significant
elevation was further shown for 6 cytokines in both right and
left eye of aniridia patients compared with controls. These six
were: IL-1β, II-9, IL-17A, eotaxin, FGF2, and MIP-1α. No
significant differences were found between right and left eye
within the aniridia and patient group.

The level of IL-1RA was lower in aniridia in all test groups,
but the differences were not statistically significant. However,
the ratio between IL-1RA and IL-1β was significantly lower in
the aniridia group in both eyes and with both Schirmer and
capillary test: in COD 17,086 ± 24,555 in aniridia versus
163,852 ± 308,989 in controls (P = 0.005); in SOS 8890 ±
20,092 versus 104,790 ± 127,283 (P = 0.001); and in COS 749
± 1611 versus 2238 ± 3085 (P = 0.05). The difference in the
IL-1RA/IL-1β ratio was not significant in COD (971 ± 1297
versus 4902 ± 5707, P = 0.07).

Table 1. Number of Tear Samples and Corresponding Age in Each Test Group

<table>
<thead>
<tr>
<th>Test</th>
<th>Aniridia, n</th>
<th>Control, n</th>
<th>Aniridia, Age, y</th>
<th>Control, Age, y</th>
</tr>
</thead>
<tbody>
<tr>
<td>Schirmer OD</td>
<td>27</td>
<td>19</td>
<td>36.2</td>
<td>29.4</td>
</tr>
<tr>
<td>Schirmer OS</td>
<td>26</td>
<td>17</td>
<td>37.5</td>
<td>29.2</td>
</tr>
<tr>
<td>Capillary OD</td>
<td>22</td>
<td>18</td>
<td>37.9</td>
<td>30.4</td>
</tr>
<tr>
<td>Capillary OS</td>
<td>19</td>
<td>13</td>
<td>36.3</td>
<td>32.2</td>
</tr>
</tbody>
</table>

* Mean.

Table 2. Tear Cytokines in Right Eye With Significantly Different Concentrations in Aniridia and Control Group

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Aniridia</th>
<th>Control</th>
<th>P Value *</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1β Schirmer test (S)</td>
<td>10.3 ± 17.2</td>
<td>1.8 ± 5.4</td>
<td>0.0001</td>
</tr>
<tr>
<td>IL-1β capillary test (C)</td>
<td>13.3 ± 8.7</td>
<td>4.5 ± 3.3</td>
<td>0.00005</td>
</tr>
<tr>
<td>IL-2 (C)</td>
<td>15.9 ± 13.7</td>
<td>4.1 ± 5.9</td>
<td>0.002</td>
</tr>
<tr>
<td>IL-9 (S)</td>
<td>6.5 ± 5.0</td>
<td>2.5 ± 3.8</td>
<td>0.001</td>
</tr>
<tr>
<td>IL-9 (C)</td>
<td>35.3 ± 19.3</td>
<td>16.8 ± 12.7</td>
<td>0.001</td>
</tr>
<tr>
<td>IL-17A (S)</td>
<td>20.7 ± 27.0</td>
<td>5.5 ± 21.1</td>
<td>0.0004</td>
</tr>
<tr>
<td>IL-17A (C)</td>
<td>105.8 ± 111.9</td>
<td>2.4 ± 10.2</td>
<td>0.00003</td>
</tr>
<tr>
<td>Eotaxin (C)</td>
<td>88.9 ± 47.7</td>
<td>52.5 ± 27.2</td>
<td>0.002</td>
</tr>
<tr>
<td>FGF2 (S)</td>
<td>65 ± 54.4</td>
<td>19.4 ± 74.5</td>
<td>0.0002</td>
</tr>
<tr>
<td>FGF2 (C)</td>
<td>189.3 ± 150.6</td>
<td>10.5 ± 43.9</td>
<td>0.00003</td>
</tr>
<tr>
<td>MIP-1α (S)</td>
<td>1.8 ± 1.4</td>
<td>0.7 ± 1.7</td>
<td>0.001</td>
</tr>
<tr>
<td>MIP-1α (C)</td>
<td>13.0 ± 6.4</td>
<td>7.1 ± 4.7</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Mean ± SD: cytokines (pg/mL).
* Mann-Whitney U test and Holm-Bonferroni method.
One of the cytokines that were significantly elevated in both eyes were IFN-γ (S) 4.8 ± 0.00001, Eotaxin (S) 15.0 ± 4.9, IL-13 (S) 15.0 ± 4.9, IL-10 (S) 14.2 ± 4.9, and IL-9 (S) 6.0 ± 4.9. These cytokines were significantly higher in controls than in the aniridia group. However, the ratio between the anti-inflammatory IL-1RA and the pro-inflammatory IL-1β was significantly higher in controls. The levels of six cytokines were significantly elevated in both eyes in aniridia (IL-1β, IL-9, IL-17A, eotaxin, FGF2, and MIP-1α). Bilateral confirmation of these six cytokines makes it reasonable to consider them as particularly important among the tested proteins.

Several studies have successfully used multiplex bead analysis in examination of tear fluid.12–14 Smaller sample volumes are needed with this method than traditional procedure—ELISA—thus both eyes could be tested separately without need to pool samples.15 Furthermore, multiplex analysis has good correlation with ELISA test, rapid processing and high throughput, which allows quantification of multiple protein targets.15

A different number of cytokines with significantly higher concentrations was detected with Schirmer test and capillary test from the same eye, and in the right and left eyes. This might reflect variability in expression and severity of disease between the two eyes in a patient with aniridia, and the ability to detect these differences by Schirmer and capillary test. On the other hand, the Schirmer strip was always first inserted into right inferior fornix, and the capillary test started with right eye, which could have influenced the results. Moreover, the number of samples achieved was higher with Schirmer test than with capillary test, and higher in right eye than left eye. Cytokine levels in the Schirmer test groups correlated better with clinical parameters than those from capillary test groups. Our study therefore indicates that Schirmer test from one eye should be chosen when collecting tear samples for cytokine analyses. Exclusion of Schirmer tests with 5 mm wetting or less may have impacted our results by excluding participants with more severe DED.

Several cytokines have been identified in tear fluid from normal individuals.15 Both proinflammatory and anti-inflammatory cytokines are present in normal eyes without inflammation,16 and some of these are suggested to participate in the host defense system at the ocular surface.15 Elevated concentration of tear fluid cytokines has been shown in patients harboring systemic diseases which affect the ocular surface, such as graft-versus-host-disease,17 and in inflammatory eye diseases involving the ocular surface.18

Increased level of many inflammatory cytokines has been evident in patients with DED.6,12 The prevalence of DED in the aniridia is high, and correlates with severity of AAK. AAK could lead to corneal ulceration and inflammation,19 and be a sight threatening complication in aniridia.

Elevated concentrations of IL-1β have been found in tear fluid from DED patients,6,14 and recognized as the earliest change in patients with DED.14 In our study, higher concentration of IL-1β was associated with lower age in the aniridia group. This may reflect that increased level of IL-1β is an initial
MGD.21 production is probably also a compensatory mechanism in aniridia patients. Raised tear concentration of a number of cytokines. This might reflect increasing Schirmer test values corresponded with higher and parameters for DED, including tests for MGD. Interestingly, correlations were found between the level of several cytokines increased in both eyes in our study. Additionally, significant correlations were demonstrated between cytokines and parameters for DED. IL-1β plays a role in the pathogenesis of DED. In a study by Enriquez-de-Salamanca et al., increased levels of inflammatory cytokines were found in patients with moderate forms of evaporative DED due to MGD. Moreover, correlations were demonstrated between cytokines and clinical parameters for DED. In aniridia, MGD has been identified as a possible factor in the development of DED in most patients. It is likely that MGD contributes to the tear cytokine profile demonstrated in aniridia patients. In our study, this argument is supported by moderate to strong correlations between elevated cytokine concentrations and increased atrophy of MGs in terms of meiboscore. Furthermore, positive lipography, indicating maintained tear film lipid layer, was associated with decreased level of IL-9 and IL-17A. Longer break-up time of the tear film, implying better function of the tear lipid layer, was associated with lower concentration of IL-17A and FGF2 and hence less inflammation. Additionally, the presence of eyelid and conjunctival pathologies correlated positively with the level of cytokines in certain subgroups.

Eyelid changes could potentially contribute to the development of MGD, or possibly be a consequence of the disease. Clinically, corneal ulceration and inflammation are frequently observed in aniridia patients, and the presence of inflammatory cells in the corneal limbal region was demonstrated by laser scanning in vivo confocal microscopy. Confocal microscopy also showed inflammatory cells in the central cornea despite transparency by slit-lamp examination. Thus, it is likely that corneal processes contribute to inflammation at the ocular surface and increase the concentration of inflammatory substances in the tear film. Correlation between stage of AAK and cytokine level was not shown in our study, possibly because the number of participants was not large enough for subgroup analysis. In future studies, cytokine levels should be analyzed in each group of AAK if enough patients exist.

Proinflammatory properties characterize all six cytokines that were significantly elevated in both eyes (IL-1β, IL-9, IL-17A, eotaxin, FGF2, and MIP-1α). The expression of IL-1β is increased in the tissue covering cornea (pannus) in limbal stem cell deficiency. Furthermore, highly elevated expression of IL-9 has been found in conjunctival cells of patients with DED connected to graft versus host disease. Th17 cells mediate ocular surface autoimmunity through IL-17A. Eotaxin secreted in tears is important in the pathogenesis of seasonal allergic conjunctivitis. Moreover, increased expression of eotaxin was shown in tears and conjunctival epithelial cells of patients with vernal keratoconjunctivitis. Upregulation of FGF2 in tear fluid has been found in cases of chronic Stevens-Johnson syndrome. Furthermore, an elevated level of MIP-1α/CCL3 was demonstrated together with increasing degree of

### Table 4. Correlation Analysis Between Levels of Five Selected Tear Cytokines and Clinical Parameters in the Aniridia Group

<table>
<thead>
<tr>
<th>Clinical Parameter</th>
<th>Test Group</th>
<th>Cytokine</th>
<th>Correlation Coefficient*</th>
<th>P Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>SOD</td>
<td>IL-1β</td>
<td>-0.485</td>
<td>0.01</td>
</tr>
<tr>
<td>Tear production</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tear meniscus height</td>
<td>SOS</td>
<td>MIP-1α</td>
<td>-0.450</td>
<td>0.02</td>
</tr>
<tr>
<td>Schirmer test</td>
<td>SOD/SOS</td>
<td>IL-1β</td>
<td>0.622/0.618</td>
<td>0.001/0.001</td>
</tr>
<tr>
<td></td>
<td>SOD</td>
<td>IL-9</td>
<td>0.519</td>
<td>0.006</td>
</tr>
<tr>
<td></td>
<td>SOD</td>
<td>IL-17A</td>
<td>0.382</td>
<td>0.049</td>
</tr>
<tr>
<td></td>
<td>SOD</td>
<td>FGF2</td>
<td>0.425</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>SOD</td>
<td>MIP-1α</td>
<td>0.588</td>
<td>0.001</td>
</tr>
<tr>
<td>Phospholipid test</td>
<td>SOS</td>
<td>FGF2</td>
<td>0.821</td>
<td>0.02</td>
</tr>
<tr>
<td>Tear film quality</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Break-up time</td>
<td>SOD</td>
<td>IL-17A</td>
<td>-0.457</td>
<td>0.03</td>
</tr>
<tr>
<td>Lipography (positive)</td>
<td>SOS</td>
<td>IL-9</td>
<td>-0.400</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>SOS</td>
<td>IL-17A</td>
<td>-0.423</td>
<td>0.03</td>
</tr>
<tr>
<td>Eyelid</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Telangiectasia eyelid</td>
<td>SOS</td>
<td>FGF2</td>
<td>0.522</td>
<td>0.006</td>
</tr>
<tr>
<td>Meiboscore</td>
<td>SOD/COD</td>
<td>IL-1β</td>
<td>0.488/0.477</td>
<td>0.04/0.04</td>
</tr>
<tr>
<td>Lower eyelid</td>
<td>SOD/SOS</td>
<td>IL-9</td>
<td>0.488/0.516</td>
<td>0.02/0.01</td>
</tr>
<tr>
<td></td>
<td>SOD/COD/SOS</td>
<td>IL-17A</td>
<td>0.470/0.457/0.460</td>
<td>0.03/0.049/0.03</td>
</tr>
<tr>
<td></td>
<td>COD/SOS</td>
<td>FGF2</td>
<td>0.464/0.452</td>
<td>0.04/0.04</td>
</tr>
<tr>
<td></td>
<td>COD</td>
<td>MIP-1α</td>
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* Spearman’s rank correlation.
FIGURE. Scatter plot of correlation between clinical parameters and concentration of various inflammatory tear cytokines collected with Schirmer test in right eye: (A) correlation between Schirmer test results (mm) and concentration of IL-1β (pg/mL); (B) between Schirmer test results and MIP-1α; (C) tear film break-up time (s) and FGF2; (D) tear film break-up time and IL-17A; (E) meiboscore and IL-9; (F) meiboscore and IL-1β. R and P values calculated with Spearman’s rank correlation.
neutrophilic cells in a corneal model.\textsuperscript{32} MIP-1\textalpha/CCL3 represents an important signal for mast cell degranulation in the conjunctiva and hence for acute-phase disease.\textsuperscript{35}

The proinflammatory IL-1\beta is an agonist and the anti-inflammatory IL-1RA an antagonist to the same receptors, and the balance between IL1 and IL-1RA impacts the development of inflammatory diseases.\textsuperscript{54} In our study, the ratio between IL-1RA and IL-1\beta was significantly reduced in aniridia. This finding indicates an imbalance between pro- and anti-inflammatory cytokines in aniridia patients, and suggests that a restoration of this cytokine balance (which exists in tears from healthy subjects) could be a future therapeutic goal.

Research points to inflammatory events at the ocular surface as a core mechanism in the development of DED.\textsuperscript{7,35} AAK probably enhances these events and serves as a possible trigger for DED in aniridia patients. Moreover, DED potentially leads to ocular surface epithelial disease and altered corneal epithelial barrier function.\textsuperscript{7} Accordingly, AAK and DED may interact, progressively breaking down the normal corneal anatomy and reducing the patient’s vision.

In conclusion, our study demonstrates significantly higher levels of a number of inflammatory cytokines in tear fluid from aniridia patients than in control individuals. Moderate correlations were found between cytokine concentrations and parameters for DED, especially MGD. Increased inflammation of the ocular surface may in part explain the high prevalence of DED in aniridia. In turn, MGD and hence DED possibly contribute to elevated cytokine levels in the tear fluid. As inflammation may lead to deterioration of AAK and consequently the patient’s vision, attention should be directed toward possible treatment options targeting these disease pathways.

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