Glaucoma

# Cytokine and Growth Factor Analysis in Exfoliation Syndrome and Glaucoma

Do Young Park, Moohyun Kim, and Soon Cheol Cha

Department of Ophthalmology, Yeungnam University College of Medicine, Yeungnam University Hospital, Daegu, Korea

PURPOSE. We compared cytokines, chemokines, and growth factors in the aqueous humor (AH) of patients with exfoliation syndrome (XFS), with exfoliation glaucoma (XFG), with primary open angle glaucoma (POAG), and healthy controls.

METHODS. AH samples were collected from 21 patients with XFS, 28 with XFG, 14 with POAG, and 17 healthy controls during routine cataract surgery. The protein levels of 21 cytokines and growth factors, together with TGF-$\beta$1, 2, and 3, were quantified using the multiplex immunoassay. The levels of each protein in the four groups were compared using the Kruskal-Wallis test.

RESULTS. Among the 24 cytokines and growth factors, 16 were out of the detectable range in >50% of samples in at least one group; the remaining 8 cytokines and growth factors (IL-8, MIP-1$\alpha$, fractalkine, Flt3 ligand, PDGF-AA, VEGF, TGF-$\beta$1, and TGF-$\beta$2) were included in the analysis. TGF-$\beta$1 and TGF-$\beta$2 levels were the highest in patients with XFG and those with POAG, respectively. Expression levels of the inflammatory chemokines IL-8, MIP-1$\alpha$, and fractalkine, as well as levels of the immune cell growth factor Flt3 ligand, were significantly higher in the XFG group than in the other groups. The protein levels of PDGF-AA and VEGF were not significantly different among the 4 groups.

CONCLUSIONS. Both TGF-$\beta$1 and inflammatory cytokines were highly expressed in the AH of patients with XFG. Considering that the levels of these cytokines are increased by oxidative stress and that they regulate the extracellular matrix, they may also play a role in intraocular pressure elevation in XFG.

Keywords: exfoliation syndrome, exfoliation glaucoma, aqueous humor, cytokine, growth factor

Exfoliation syndrome (XFS) is an age-related disease characterized by the abnormal accumulation of extracellular fibrillar material in the anterior segment of the eye.[1–3] Exfoliative material is usually found on the pupillary border and anterior lens capsule, and it may elevate intraocular pressure (IOP) by various mechanisms, which leads to the development of exfoliation glaucoma (XFG). The deposition of exfoliative material on the trabecular meshwork (TM) leads to blockage of aqueous outflow, juxtacanalicular endothelial cell dysfunction, and alterations in protein expression in the aqueous humor (AH).[1,3]

The AH contains electrolytes, organic solutes, growth factors, and cytokines that maintain the metabolic balance of the anterior segment of the eye.[4,5] Homeostasis of the AH can be disrupted in several ocular diseases, which leads to elevated expression of several growth factors and cytokines. Transforming growth factor (TGF)-$\beta$1[6,7] and connective tissue growth factor (CTGF)[8–10] are known to be expressed at higher levels in the AH of patients with XFG than in the AH of normal controls. These growth factors are involved in the production of exfoliative materials or alterations in cells involved in the outflow facility, resulting in increased outflow resistance. Conversely, increased exfoliative material or IOP elevation may lead to the secondary production of several growth factors or inflammatory cytokines in the AH. These can also be interconnected and create a vicious cycle.

Therefore, determining which growth factors and cytokines show increased levels in the AH of patients with XFS and those with XFG can provide insightful information for studying the pathophysiology of XFG-associated IOP elevation. These proteins could also be used as biomarkers to predict the risk of XFG development from XFS.

Recently, several studies have analyzed the proteins in the AH of patients with XFS and those with XFG[11–14]; however, only few studies have simultaneously compared growth factors and cytokines in patients with XFG to those in patients with XFS, patients with primary open angle glaucoma (POAG), as well as controls.[15] In this study, we evaluated and analyzed several growth factors and cytokines in the AH of patients with XFS, with XFG, and with POAG and compared them with those in the AH of healthy controls. As such, we aimed to identify which proteins could be utilized as biomarkers specific to XFS or XFG and deduce the role of these proteins in the development of XFG.

## METHODS

### Study Subjects

This prospective study of patients XFS, with XFG, and with POAG and healthy controls was approved by the Yeungnam University Hospital Institutional Review Board (IRB)

Investigative Ophthalmology & Visual Science

and followed the tenets of the Declaration of Helsinki (IRB No. 2020-04-124). This study was performed with the prior written consent of each participant.

AH samples with volumes of approximately 50 to 100 µL were obtained from 21 patients with XFS, 28 patients with XFG, 14 patients with POAG, and 17 healthy controls at the onset of routine phacoemulsification surgery from April 2020 to June 2021 at Yeungnam University Hospital. The samples were immediately transferred to a −80°C deep freezer and stored until analysis.

XFG was defined according to the following criteria: (1) untreated IOP >21 mm Hg, (2) open angle on gonioscopy, (3) glaucomatous optic disc change and corresponding glaucomatous visual field defect, and (4) presence of exfoliation material observed at the anterior lens capsule and/or at the pupillary border. XFS was defined by the presence of exfoliation material at the anterior lens capsule in patients with a normal optic disc and normal IOP. POAG was defined as those cases where criteria (1), (2), and (3) were satisfied, and where there was no exfoliation material in the eye.[16]

Individuals with other intraocular disorders, such as diabetic retinopathy, those with uncontrolled IOP (>21 mm Hg), those who underwent trabeculectomy or Ahmed valve implantation within 6 months, and those who had used anti-inflammatory eye drops within 6 months before cataract surgery were excluded.

Data on the demographics, preoperative IOP, and the number of currently using glaucoma eyedrops were collected from the participants.

## Cytokine and Growth Factor Analyses

A magnetic multiplex kit for 21 human cytokines and growth factors (Human Growth Factor Magnetic Luminex Performance Assay 21-plex Fixed Panel) and a kit for TGF-$\beta$1, 2, and 3 (Magnetic Luminex Performance Assay TGF-$\beta$ Base Kit) were purchased from R&D Systems (Minneapolis, MN, USA) and used according to the manufacturer's instructions were purchased from R&D Systems (Minneapolis, MN, USA) and used according to the manufacturer's instructions. The following cytokines and growth factors were tested in these assays: macrophage inflammatory protein (MIP)-1$\alpha$, MIP-3$\beta$, CD40 ligand, fractalkine, MIP-2$\alpha$, epidermal growth factor (EGF), fibroblast growth factor (FGF)2, Fms-related tyrosine kinase (Flt)3 ligand, granulocyte colony-stimulating factor (G-CSF), granzyme B, interferon (IFN)-$\beta$, interleukin (IL)-3, IL-8, IL-25, and IL-33, programmed cell death-ligand 1 (PD-L1), PDGF-AA, PDGF-AB/BB, transforming growth factor (TGF)-$\alpha$, TNF-related apoptosis-inducing ligand (TRAIL), vascular endothelial growth factor (VEGF), TGF-$\beta$1, TGF-$\beta$2, and TGF-$\beta$3.

AH samples were added to a mixture of fluorescently labeled magnetic microspheres coupled with analyte-specific capture antibodies. After the addition of biotinylated detection antibodies and phycoerythrin (PE)-conjugated streptavidin, fluorescence was quantified using the Bio-Plex 200 system (Bio-Rad, Hercules, CA, USA). Data were analyzed using the Bio-Plex Manager software (Bio-Rad). Cytokine and growth factor levels were reported as mass per volume (pg/mL). Cytokines were excluded from the analyses when more than 50% of the values were outside the upper or lower limits of detection. If the value was less than the lowest detection value, 50% of the lowest detection value was applied.[17]

## Statistical Analysis

The Shapiro-Wilk test was used to check whether the data were normally distributed. For comparison of continuous variables, such as cytokine concentrations or age between groups, the Kruskal-Wallis test was performed with a post hoc analysis using Dunn's test. Spearman's correlation test was used to calculate the correlations between the concentration of each cytokine and growth factor, age of the participant, and IOP. The level of statistical significance was set at $P < 0.05$. Statistical analysis was performed using the R statistical package version 3.5.3 (R Foundation for Statistical Computing, Vienna, Austria).

## RESULTS

The characteristics of the participants are summarized in Table 1. The mean age of the participants was 72.3 (standard deviation [SD]: 8.4), and 47.5% participants were men. Age, sex, and IOP were not significantly different among the four groups ($P > 0.05$). The number of IOP-lowering medications taken was not different between the POAG and XFG groups ($P > 0.05$).

Of the 24 cytokines and growth factors, 16 (CD40 ligand, EGF, FGF2, G-CSF, granzyme B, MIP-2$\alpha$, IFN-$\beta$, IL-3, IL-25, IL-33, MIP-3$\beta$, PDGF-AB/BB, PD-L1, TRAIL, TGF-$\alpha$, and TGF-$\beta$3) were out of the detection range in >50% of samples in at least one group. The remaining eight cytokines and growth factors (IL-8, MIP-1$\alpha$, fractalkine, Flt3 ligand, PDGF-AA, VEGF, TGF-$\beta$1, and TGF-$\beta$2) were included in the analysis (Supplementary Table S1).

In the intergroup comparison (Table 2, Fig. 1), TGF-$\beta$1 concentration was significantly higher in patients with XFG than in the control group ($P = 0.002$). In contrast, TGF-$\beta$2 concentration was the highest in patients with POAG. The expression of IL-8, MIP-1$\alpha$, fractalkine, and Flt3 ligands was significantly higher in patients with XFG than in the other three groups. The protein levels of PDGF-AA and VEGF were not significantly different among the four groups ($P > 0.05$).

In the correlation analysis (Fig. 2, Supplementary Table S2), the inflammatory chemokines IL-8, MIP-1$\alpha$, and

**TABLE 1.** Demographic and Clinical Characteristics of the Study Participants

| Variables | Normal | POAG | XFS | XFG | P Value |
|---|---|---|---|---|---|
| Eyes, *n* | 17 | 14 | 21 | 28 | |
| Sex, male/female | 8/9 | 7/7 | 8/13 | 16/12 | 0.63 |
| Age, years | 70.1 ± 9.7 | 71.0 ± 6.6 | 72.3 ± 7.6 | 74.3 ± 8.7 | 0.31 |
| IOP, mmHg | 13.8 ± 3.2 | 13.9 ± 1.8 | 15.0 ± 3.1 | 13.3 ± 2.7 | 0.25 |
| Number of eyedrops | 0 | 1.8 ± 0.5 | 0 | 1.5 ± 1.2 | 0.27 |

Data are presented as mean ± standard deviation (SD).
*P* values were calculated using the Kruskal-Wallis test followed by Dunn's multiple comparison test.

**TABLE 2.** Quantification and Comparison of the Concentrations of Cytokines and Growth Factors in the Aqueous Humor by Group

| | Groups | | | | P Value | | | | | |
| | Normal (n = 17) | POAG (n = 14) | XFS (n = 21) | XFG (n = 28) | Normal Versus POAG | Normal Versus XFS | POAG Versus XFS | Normal Versus XFG | POAG Versus XFG | XFS Versus XFG |
|---|---|---|---|---|---|---|---|---|---|---|
| IL-8 | 6.44 ± 3.82 | 9.27 ± 7.67 | 6.14 ± 3.29 | 21.75 ± 20.68 | 0.446 | 0.917 | 0.454 | **7.44E-04** | **0.028** | **3.49E-04** |
| MIP-1α | 10.66 ± 5.35 | 9.82 ± 6.29 | 16.34 ± 5.48 | 21.88 ± 10.28 | 0.995 | **0.036** | 0.388 | **2.90E-05** | **5.11E-05** | **0.041** |
| Fractalkine | 81.09 ± 16.01 | 80.05 ± 21.97 | 112.10 ± 38.04 | 170.45 ± 71.50 | 0.982 | **0.037** | **0.042** | **2.31E-06** | **6.28E-06** | **8.25E-03** |
| Flt-3 ligand | 2.52 ± 3.76 | 4.38 ± 5.16 | 4.65 ± 3.70 | 12.14 ± 11.67 | 0.517 | 0.219 | 0.582 | **7.81E-04** | **0.019** | **0.029** |
| TGF-β1 | 13.69 ± 10.69 | 43.71 ± 42.93 | 59.84 ± 67.77 | 201.53 ± 340.84 | 0.376 | 0.190 | 0.696 | **2.43E-03** | 0.081 | 0.082 |
| TGF-β2 | 799.97 ± 270.15 | 1239.01 ± 452.69 | 680.71 ± 289.74 | 926.36 ± 523.73 | **0.034** | 0.257 | **8.01E-04** | 0.699 | **0.030** | 0.104 |
| PDGF-AA | 97.70 ± 31.42 | 101.22 ± 32.52 | 107.88 ± 36.68 | 124.59 ± 44.78 | 0.844 | 0.523 | 0.595 | 0.167 | 0.193 | 0.399 |
| VEGF | 95.26 ± 71.82 | 79.48 ± 32.79 | 103.80 ± 47.38 | 114.60 ± 55.49 | 0.920 | 0.226 | 0.287 | 0.139 | 0.0753 | 0.513 |

Data are presented as mean (pg/mL) ± standard deviation (SD).

*P* values were calculated using the Kruskal-Wallis test followed by Dunn's multiple comparison test.

Statistically significant values are shown in bold.

IL, interleukin; MIP, macrophage inflammatory protein; Flt, Fms-related tyrosine kinase; TGF, transforming growth factor; PDGF, platelet derived growth factor; VEGF, vascular endothelial growth factor.
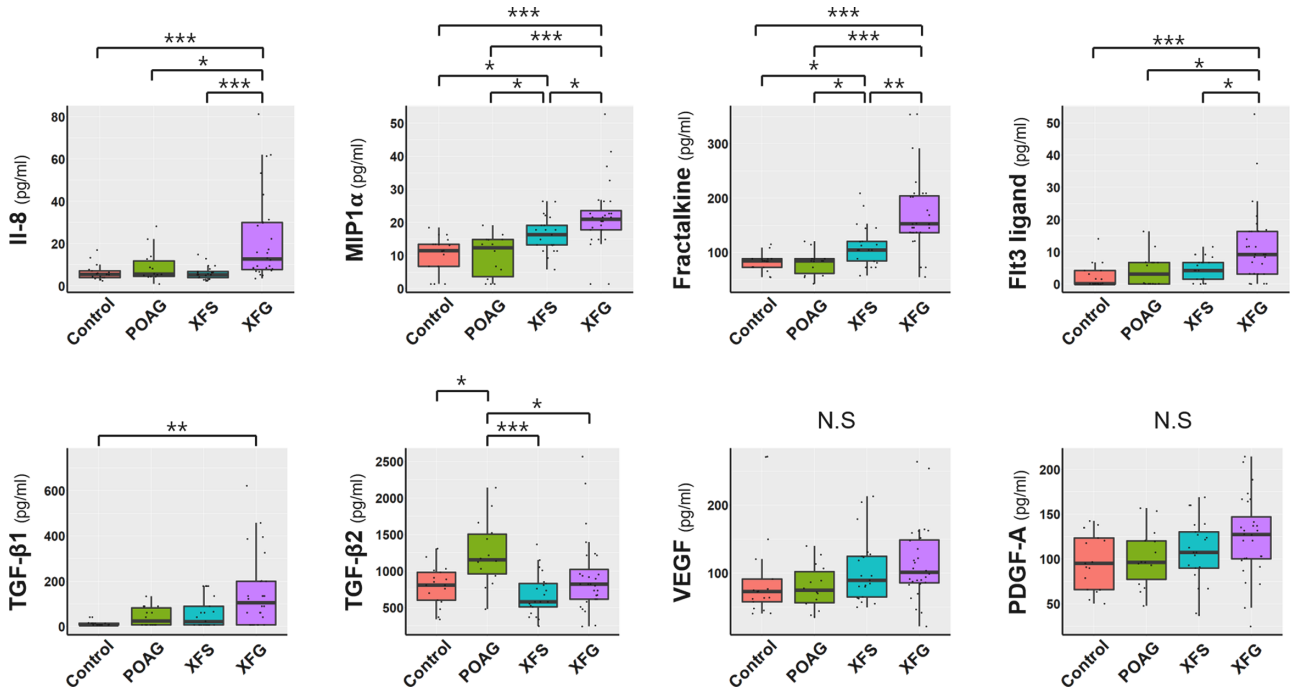
**FIGURE 1.** Levels of cytokines and growth factors in the aqueous humor, as determined using a multiplex immunoassay. *P < 0.05, **P < 0.01, ***P < 0.001 by Kruskal-Wallis test followed by Dunn's multiple comparison test.
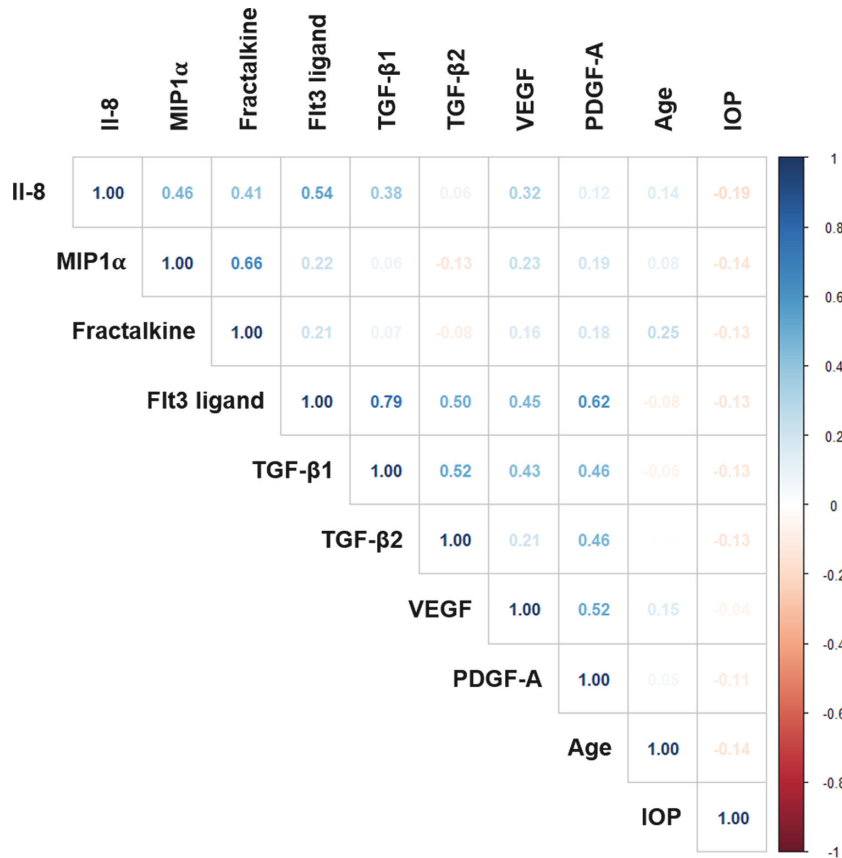


**FIGURE 2.** Correlation matrix showing correlation coefficients between the concentrations of each cytokine and growth factor in the aqueous humor, age of the participant, and intraocular pressure. The correlation coefficient is displayed in blue in cases where the correlation coefficient was closer to 1.

fractalkine showed positive correlations with each other. Similarly, the growth factors TGF-$\beta$1, TGF-$\beta$2, PDGF-AA, and VEGF were strongly and positively correlated with each other. Among inflammatory cytokines, the Flt3 ligand was correlated with other growth factors, whereas among growth factors, VEGF was correlated with other inflammatory chemokines. Age was not correlated with cytokines and growth factors, except for fractalkine. IOP was not correlated with any of the cytokines or growth factors.

## DISCUSSION

This study demonstrates that inflammatory cytokines and chemokines, such as IL-8, MIP-1$\alpha$, fractalkine, and Flt3 ligand were specifically and highly expressed in the AH of patients with XFG, in addition to the previously known TGF-$\beta$1.[6] The levels of all these proteins were significantly increased in patients with XFG as compared to those in healthy controls, patients with XFS, and those with POAG. The results suggest that these inflammatory cytokines and chemokines and TGF-$\beta$1 are involved in the development of XFG.

The significant increase in the levels of these inflammatory cytokines and chemokines in the AH of patients with XFG suggests that XFG may be a low-grade inflammatory condition. The presence of inflammatory cytokines can induce various pathological conditions of the eye through direct or indirect regulation of extracellular matrix (ECM) remodeling, vascular permeability, and IOP.[2] In fact, inflammatory cytokines are known to cause ECM remodeling in various diseases, such as cancer and lung fibrosis.[18,19] In addition, studies have revealed that the treatment of various cells, including ciliary epithelial cells, with cytokines induces the expression of ECM molecules.[15,20] In this regard, increased levels of inflammatory cytokines in patients with XFG compared to those in patients with XFS, those with POAG, and controls suggest that the accumulation of exfoliation-related low-grade inflammatory factors may promote TM cell remodeling by inducing ECM expression, leading to elevated IOP and the development of XFG.

In this study, the cytokines that were detected in more than 50% of the samples of all 4 groups were IL-8, MIP-1$\alpha$, fractalkine, and Flt3 ligand, all of which showed significantly higher expression in patients with XFG than in normal controls and patients with POAG or XFS. IL-8 is a CXC chemokine that attracts neutrophils, basophils, and T cells. It also plays a role in neutrophil recruitment and degranulation. Its expression is increased by oxidative stress, and it is released from several cell types in response to inflammation.[21] In cultured human TM cells, increased secretion of IL-8 has been reported in response to chronic oxidative stress or pro-inflammatory cytokines, such as tumor necrosis factor (TNF)-$\alpha$ and IL1-$\beta$.[22,23] MIP-1$\alpha$ is a member of the CC chemokine subfamily. It is secreted by various mature hematopoietic cell types, and it functions as a mediator for the recruitment and activation of macrophages.[24] It is known to mediate macrophage-induced wound healing by increasing collagen synthesis and angiogenic activity.[25] One study reported that MIP-1$\alpha$ expression increased with age, and another study reported that MIP-1$\alpha$ expression was increased in the TM tissue of patients with POAG.[26,27] Fractalkine is a unique chemokine existing in a soluble form or a membrane-bound form. Soluble fractalkine is associated with the inflammatory cascade and hence serves as an inflammatory marker.[28] It also exhibits chemo-attractive

activity for monocytes, natural killer cells, and T cells.[29] Flt3 ligand is a hematopoietic cytokine that acts as the main growth factor for dendritic cell differentiation.[30] Studies on the expression and role of fractalkine and the Flt3 ligand in eyes are limited. However, in mesangial cells, fractalkine induces the expression of TGF-$\beta$1 and ECM molecules, such as fibronectin, collagen type 1, and collagen type 4.[31] In addition, the protein level of Flt3 ligand was found to be increased in fibrotic lung tissue, and the Flt3 ligand-induced increase in dendritic cell numbers was found to be associated with increased expression of ECM-degrading enzymes, suggesting the possibility that the Flt3 ligand regulates fibrosis.[19] Overall, the increase in levels of these inflammatory cytokines and chemokines in the AH serves as a biomarker of XFG indicative of a low-grade inflammatory state; it also implies that expression of these cytokines may be increased in response to oxidative stress, increasing outflow resistance by actively regulating the ECM of TM cells, thereby causing IOP elevation in XFG.

The levels of these inflammatory cytokines increase in proportion to the extent of damage to the blood aqueous barrier (BAB), which is critical for the development of XFS and XFG. Conversely, an increase in levels of these inflammatory cytokines may further damage the BAB. The BAB is mainly made up of tight junctions in the non-pigmented ciliary epithelium and endothelial cells in the iris.[32] Various proteins and the ECM in serum can accumulate in the AH due to damage to the vasculature constituting the BAB, and exfoliative materials are produced by abnormal aggregation of these proteins.[2,33] Genes related to XFS, such as those encoding fibrillin, elastin, and LOXL1, are expressed in blood vessels, including those of the eyes.[20,34] Furthermore, early-onset XFS is related to a history of previous ocular surgery damaging the iris, which supports the significance of BAB disruption in the pathogenesis of XFS.[33] Damage to the BAB is closely related to oxidative stress, markers of which are known to show increased levels in the AH of patients with XFS.[35] In addition, oxidative stress is known to induce fibrosis by triggering TGF-$\beta$ expression and ECM synthesis; it also increases vascular permeability by inducing endothelial barrier dysfunction.[36] Accordingly, the high levels of inflammatory cytokines in patients with XFG in this study suggest that damage to the BAB and oxidative stress are the most severe in patients with XFG and that these factors may lead to a vicious cycle in which exfoliative materials increase outflow resistance.

Regarding VEGF, there is a discrepancy between the results of studies. One study reported that the VEGF level was higher in patients with XFG and those with POAG than in controls, but no difference was observed between patients with XFG and those with POAG.[37] Another study reported that both patients with XFS and those with XFG showed higher VEGF concentrations than the controls.[38] However, one study reported no differences in VEGF levels between controls, patients with POAG, and those with XFG.[11] It is still unclear whether the level of VEGF reflects the ischemic intraocular condition due to high IOP or if it is specific to XFG or POAG. In this study, the VEGF concentration was not significantly different among the XFS, XFG, POAG, and control groups. These results might be related to the fact that there was no difference in IOP in the groups in this study, and VEGF might not be directly involved in the development of XFG or POAG. However, the VEGF level was positively correlated with IL-8, MIP-1$\alpha$, Flt3 ligand, PDGF-AA, TGF-$\beta$1, and TGF-$\beta$2 levels, suggesting that VEGF plays a role

in regulating inflammatory cytokines or fibrogenic growth factors, which may indirectly affect the characteristics of the TM.

This study has several limitations. First, the number of AH samples in each group was small, thereby potentially causing a type 2 statistical error in the comparison of some cytokines between groups. Second, although we tried to evaluate as many cytokines and growth factors as possible with small volumes of AH using a multiplex system, a number of cytokines and growth factors were not detected in most of the control samples, making it impossible to compare these substances among groups. The creation of a new panel, comprising cytokines that are highly expressed in XFG specifically and are well detected in normal controls, could help identify novel cytokine expression patterns that can be utilized in the diagnosis and determination of prognosis of XFG in the future. Third, even though the IOP was not significantly correlated with the elevated levels of cytokines in the AH of patients with XFG in this study, we cannot overlook the fact that IOP may affect the levels of cytokines because we did not include patients with an uncontrolled IOP. In addition, although the number of IOP-lowering eyedrops was not significantly different between the POAG and XFG groups, eyedrop use might affect the levels of cytokines in the AH.[39,40] Further studies including patients with a high IOP with and without eyedrop use may help to determine the effect of both IOP elevation and eyedrop use on the levels of cytokines in the AH of patients with XFG. Fourth, we did not evaluate clinical characteristics, such as baseline IOP, visual field defect, or optic disc parameters in relation to the concentration of each cytokine. This needs to be analyzed using more samples in the future. Finally, and most importantly, we only inferred the possible roles of certain cytokines and growth factors in the development of XFG by observing that they were expressed at high levels in the eyes of patients with XFG. The mechanism by which these molecules regulate IOP and affect the development of XFG should be elucidated by investigating the expression of these molecules in human tissue, in vitro experiments using TM cells, and animal studies.

In summary, we analyzed several cytokines and growth factors in the AH of patients with XFG, with XFS, and with POAG using a multiplex immunoassay. We confirmed that the expression of TGF-$\beta$1, the inflammatory chemokines IL-8, MIP-1$\alpha$, and fractalkine, and the immune cell growth factor Flt3 ligand were significantly high in patients with XFG. These inflammatory cytokines could be induced by oxidative stress and regulate the ECM; therefore, these cytokines may also play a role in increasing outflow resistance and IOP, together with fibrogenic growth factor TGF-$\beta$1, contributing to the development of XFG. Further molecular studies are needed to verify the detailed function of these cytokines in relation to the pathogenesis of XFG.

## Acknowledgments

Disclosure: **D.Y. Park**, None; **M. Kim**, None; **S.C. Cha**, None

## References

1. Ozaki M. Mechanisms of Glaucoma in Exfoliation Syndrome. *J Glaucoma.* 2018;27(Suppl 1):S83–S86.
2. Borras T. Growth Factors, Oxidative Damage, and Inflammation in Exfoliation Syndrome. *J Glaucoma.* 2018;27(Suppl 1):S54–S60.
3. Pasquale LR, Borras T, Fingert JH, Wiggs JL, Ritch R. Exfoliation syndrome: assembling the puzzle pieces. *Acta Ophthalmol.* 2016;94:e505–e512.
4. To CH, Kong CW, Chan CY, Shahidullah M, Do CW. The mechanism of aqueous humour formation. *Clin Exp Optom.* 2002;85:335–349.
5. Chowdhury UR, Madden BJ, Charlesworth MC, Fautsch MP. Proteome analysis of human aqueous humor. *Invest Ophthalmol Vis Sci.* 2010;51:4921–4931.
6. Schlotzer-Schrehardt U, Zenkel M, Kuchle M, Sakai LY, Naumann GO. Role of transforming growth factor-beta1 and its latent form binding protein in pseudoexfoliation syndrome. *Exp Eye Res.* 2001;73:765–780.
7. Yoneda K, Nakano M, Mori K, Kinoshita S, Tashiro K. Disease-related quantitation of TGF-beta3 in human aqueous humor. *Growth Factors.* 2007;25:160–167.
8. Ho SL, Dogar GF, Wang J, et al. Elevated aqueous humour tissue inhibitor of matrix metalloproteinase-1 and connective tissue growth factor in pseudoexfoliation syndrome. *Br J Ophthalmol.* 2005;89:169–173.
9. Browne JG, Ho SL, Kane R, et al. Connective Tissue Growth Factor Is Increased in Pseudoexfoliation Glaucoma. *Invest Ophthalmol Vis Sci.* 2011;52:3660–3666.
10. Ghanem AA, Arafa LF, El-Baz A. Connective tissue growth factor and tissue inhibitor of matrix metalloproteinase-2 in patients with exfoliative glaucoma. *Curr Eye Res.* 2011;36:540–545.
11. Takai Y, Tanito M, Ohira A. Multiplex cytokine analysis of aqueous humor in eyes with primary open-angle glaucoma, exfoliation glaucoma, and cataract. *Invest Ophthalmol Vis Sci.* 2012;53:241–247.
12. Garweg JG, Zandi S, Pfister IB, Skowronska M, Gerhardt C. Comparison of cytokine profiles in the aqueous humor of eyes with pseudoexfoliation syndrome and glaucoma. *PLoS One.* 2017;12:e0182571.
13. Kliuchnikova AA, Samokhina NI, Ilina IY, et al. Human aqueous humor proteome in cataract, glaucoma, and pseudoexfoliation syndrome. *Proteomics.* 2016;16:1938–1946.
14. Botling Taube A, Konzer A, Alm A, Bergquist J. Proteomic analysis of the aqueous humour in eyes with pseudoexfoliation syndrome. *Br J Ophthalmol.* 2019;103:1190–1194.
15. Zenkel M, Lewczuk P, Junemann A, Kruse FE, Naumann GO, Schlotzer-Schrehardt U. Proinflammatory cytokines are involved in the initiation of the abnormal matrix process in pseudoexfoliation syndrome/glaucoma. *Am J Pathol.* 2010;176:2868–2879.
16. This paper is a guideline written by the European glaucoma Society, and the author's name is not listed in the original paper. When citing this document, it is cited without the author's name. European Glaucoma Society Terminology and Guidelines for Glaucoma, 4th Edition - Chapter 2: Classification and terminology Supported by the EGS Foundation: Part 1: Foreword; Introduction; Glossary; Chapter 2 Classification and Terminology. *Br J Ophthalmol.* 2017;101:73–127.
17. Niwamoto T, Handa T, Murase Y, et al. Cutaneous T-cell-attracting chemokine as a novel biomarker for predicting prognosis of idiopathic pulmonary fibrosis: a prospective observational study. *Respir Res.* 2021;22:181.
18. Iijima J, Konno K, Itano N. Inflammatory alterations of the extracellular matrix in the tumor microenvironment. *Cancers (Basel).* 2011;3:3189–3205.

19. Tort Tarres M, Aschenbrenner F, Maus R, et al. The FMS-like tyrosine kinase-3 ligand/lung dendritic cell axis contributes to regulation of pulmonary fibrosis. *Thorax.* 2019;74:947–957.

20. Zenkel M, Krysta A, Pasutto F, Juenemann A, Kruse FE, Schlotzer-Schrehardt U. Regulation of lysyl oxidase-like 1 (LOXL1) and elastin-related genes by pathogenic factors associated with pseudoexfoliation syndrome. *Invest Ophthalmol Vis Sci.* 2011;52:8488–8495.

21. Roebuck KA. Oxidant stress regulation of IL-8 and ICAM-1 gene expression: differential activation and binding of the transcription factors AP-1 and NF-kappaB (Review). *Int J Mol Med.* 1999;4:223–230.

22. Luna C, Li G, Liton PB, et al. Resveratrol prevents the expression of glaucoma markers induced by chronic oxidative stress in trabecular meshwork cells. *Food Chem Toxicol.* 2009;47:198–204.

23. Shifera AS, Trivedi S, Chau P, Bonnemaison LH, Iguchi R, Alvarado JA. Constitutive secretion of chemokines by cultured human trabecular meshwork cells. *Exp Eye Res.* 2010;91:42–47.

24. Menten P, Wuyts A, Van Damme J. Macrophage inflammatory protein-1. *Cytokine & Growth Factor Rev.* 2002;13:455–481.

25. DiPietro LA, Burdick M, Low QE, Kunkel SL, Strieter RM. MIP-1 alpha as a critical macrophage chemoattractant in murine wound repair. *J Clin Invest.* 1998;101:1693–1698.

26. Micera A, Quaranta L, Esposito G, et al. Differential Protein Expression Profiles in Glaucomatous Trabecular Meshwork: An Evaluation Study on a Small Primary Open Angle Glaucoma Population. *Advances in Therapy.* 2016;33:252–267.

27. Zheng Y, Rao YQ, Li JK, Huang Y, Zhao PQ, Li J. Age-related pro-inflammatory and pro-angiogenic changes in human aqueous humor. *Int J Ophthalmol.* 2018;11:196–200.

28. Ludwig A, Weber C. Transmembrane chemokines: Versatile 'special agents' in vascular inflammation. *Thrombosis and Haemostasis.* 2007;97:694–703.

29. Jones BA, Beamer M, Ahmed S. Fractalkine/CX3CL1: A Potential New Target for Inflammatory Diseases. *Molecular Interventions.* 2010;10:263–270.

30. Wilson KR, Villadangos JA, Mintern JD. Dendritic cell Flt3-regulation, roles and repercussions for immunotherapy. *Immunol Cell Biol.* 2021;99:962–971.

31. Song KH, Park J, Park JH, Natarajan R, Ha H. Fractalkine and its receptor mediate extracellular matrix accumulation in diabetic nephropathy in mice. *Diabetologia.* 2013;56:1661–1669.

32. Coca-Prados M. The blood-aqueous barrier in health and disease. *J Glaucoma.* 2014;23:S36–S38.

33. Mayro EL, Ritch R, Pasquale LR. Early-onset Exfoliation Syndrome: A Literature Synthesis. *J Glaucoma.* 2021;30:e164–e168.

34. Zenkel M, Schlotzer-Schrehardt U. Expression and regulation of LOXL1 and elastin-related genes in eyes with exfoliation syndrome. *J Glaucoma.* 2014;23:S48–S50.

35. Koliakos GG, Konstas AG, Schlotzer-Schrehardt U, et al. 8-Isoprostaglandin F2a and ascorbic acid concentration in the aqueous humour of patients with exfoliation syndrome. *Br J Ophthalmol.* 2003;87:353–356.

36. Cheresh P, Kim SJ, Tulasiram S, Kamp DW. Oxidative stress and pulmonary fibrosis. *Biochim Biophys Acta.* 2013;1832:1028–1040.

37. Hu DN, Ritch R, Liebmann J, Liu Y, Cheng B, Hu MS. Vascular endothelial growth factor is increased in aqueous humor of glaucomatous eyes. *J Glaucoma.* 2002;11:406–410.

38. Borazan M, Karalezli A, Kucukerdonmez C, et al. Aqueous humor and plasma levels of vascular endothelial growth factor and nitric oxide in patients with pseudoexfoliation syndrome and pseudoexfoliation glaucoma. *J Glaucoma.* 2010;19:207–211.

39. Konstas AG, Koliakos GG, Karabatsas CH, et al. Latanoprost therapy reduces the levels of TGF beta 1 and gelatinases in the aqueous humour of patients with exfoliative glaucoma. *Exp Eye Res.* 2006;82:319–322.

40. Bouchemi M, Roubeix C, Kessal K, et al. Effect of benzalkonium chloride on trabecular meshwork cells in a new in vitro 3D trabecular meshwork model for glaucoma. *Toxicol In Vitro.* 2017;41:21–29.