Aqueous Humor Dynamics in Exfoliation Syndrome

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Objective: To examine how aqueous humor dynamics are affected by exfoliation syndrome (XFS) with or without elevated intraocular pressure (IOP).

Methods: Eighty participants were divided into 4 groups: (1) those with XFS and ocular normotension (n=25), (2) controls with ocular normotension without XFS, age-matched to group 1 (n=25), (3) those with XFS and ocular hypertension (n=15), and (4) controls with ocular hypertension without XFS, age-matched to group 3 (n=15). Following washout of glaucoma medications, assessments were made of IOP, episcleral venous pressure, aqueous flow, outflow facility, and uveoscleral outflow. Differences were analyzed by group mean comparisons and linear regression analyses.

Results: Uveoscleral outflow was significantly decreased in individuals with XFS compared with age-matched controls and was independent of IOP. Patients with ocular hypertension (with or without XFS) exhibited decreased outflow facility compared with those with ocular normotension (with or without XFS). Aqueous flow was not affected by the level of IOP or the presence of XFS.

Conclusions: Exfoliation syndrome in normotensive and hypertensive eyes is associated with a decrease in uveoscleral outflow, whereas in hypertensive but not normotensive eyes, it is associated with reduced outflow facility.

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Exfoliation Syndrome (XFS) is an age-related disease of the extracellular matrix characterized by an accumulation of fibrillar exfoliation material on many ocular tissues.1-3 It is acknowledged by some as the most common identifiable cause of glaucoma.1,4 While data vary throughout the world, XFS has been said to account for 20% to 25% of open-angle glaucomas overall.1 The incidence of glaucoma in patients with XFS is as much as 10- to 15-fold higher than in the general population.5,6 Furthermore, the clinical prognosis for glaucoma is worse for patients with XFS. Compared with eyes with primary open-angle glaucoma, eyes with XFS generally have more severe optic disc and visual field damage on diagnosis, are less responsive to topical ocular hypotensive medication, and more often require surgical intervention.8,9 The association between XFS and glaucoma may result from the effect of XFS on intraocular pressure (IOP). The frequency of ocular hypertension (OHT) in patients with XFS has been reported to be as high as 80%.10,11 In patients with unilateral XFS, the affected eye has a higher IOP than the contralateral eye.12-14 The correlation between XFS and OHT has been attributed, at least partly, to deposition of exfoliative material by the epithelial cells of the trabecular meshwork and Schlemm's canal with subsequent degradation and obstruction of the tissues and pathway associated with conventional outflow.15-17 The amount of exfoliative material deposited in and around the trabecular meshwork has been associated with increased IOP and the presence of glaucoma.18 Resistance to outflow via the conventional pathway is increased in eyes with XFS compared with healthy eyes,19 with the effect being significant in eyes with both XFS and OHT.20 Exfoliation syndrome, however, does not appear to be associated with a change in aqueous flow.21 The effect of XFS on uveoscleral (pressure-independent) outflow has not been investigated. We conducted this study to investigate the effects of XFS on aqueous humor dynamics in the presence or absence of OHT.

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tory of chronic inflammatory eye disease; history of ocular trauma or infection within 6 months before enrollment; any abnormalities preventing reliable IOP or fluorophotometric readings; history of intraocular or ocular laser operations; history of hypersensitivity or intolerance to fluorescein, timolol, or acetazolamide; current use of any ophthalmic glucocorticoid; or history of ineffective IOP response to β-blockers or carbonic anhydrase inhibitors. This study was approved by the University of Nebraska Medical Center institutional review board. Informed consent was obtained from all participants before their enrollment in the study.

On the screening day, participants underwent a clinical ophthalmologic examination. Intraocular pressure was measured using pneumotonometry (Model 30 Classic; Reichert Ophthalmic Instruments, Depew, New York); IOPs were recorded as the mean of 3 consecutive measurements, alternating between eyes. An ophthalmologist performed a slitlamp examination and the presence of XFS was confirmed in at least 1 eye of all of the participants in the XFS group. Gonioscopy was performed and the mean of the superior, inferior, nasal, and temporal chamber angles was determined, with 0 being closed and 4 being wide open.21

Patients with a history of XFS and either OHT (n = 15) or ocular normotension (n = 25) comprised the 2 experimental groups. Healthy volunteers with ocular normotension (n = 25) and patients with OHT (n = 15) but no other remarkable ocular pathology were the 2 age-matched control groups. Following the screening examination, participants were divided into 4 groups: XFS with ocular normotension; ocular normotension without XFS; XFS with OHT; and ocular hypertension without XFS. Patients in the OHT groups had a clinical history of IOPs greater than 21 mm Hg and were found to have IOPs greater than 21 mm Hg on the screening day but may have had an IOP of 21 mm Hg or less thereafter. The OHT and ocular normotension control groups were age-matched to the XFS groups. Where applicable, the washout period of topical ocular drugs was 4 weeks for β-blockers and prostaglandin analogues, 2 weeks for α-2 agonists, and 5 days for carbonic anhydrase inhibitors.

Participants self-administered 6 drops of fluorescein solution, 2% (Alcon Laboratories, Ft Worth, Texas), topically into both eyes at 5-minute intervals beginning 6 to 10 hours before fluorophotometric scans were taken. Between 8:30 and 9:30 AM the following day, IOPs were measured. Anterior chamber depth was measured using an A-scan (PacScan 300AP Digital Biometric Ruler; Sonomed, Lake Success, New York) or a slitlamp-mounted pachymeter (Haag Streit, Mason, Ohio). The anterior chamber volume was calculated from the anterior chamber depth measurement.24 Central corneal thickness was measured using ultrasound (PacScan 300AP Digital Biometric Ruler, Sonomed) or slitlamp (Haag Streit) pachymetry. Episcleral venous pressure was measured by venomanometry (EyeTech LTD, Morton Grove, Illinois) as the mean of 3 consecutive measurements.

Fluorophotometry was performed using a scanning ocular fluorophotometer (FM-2 Fluorotron Master Ocular Fluorophotometer; OcuMetrics, Mountain View, California) as described previously.25-30 Briefly, 4 sets of duplicate scans were collected at 45-minute intervals to determine the baseline aqueous flow rate. Participants were then given either 1 drop of timolol maleate, 0.5% (Bausch & Lomb Pharmaceuticals, Tampa, Florida), topically or 250 mg of acetazolamide (Diamox; Wyeth-Ayerst, Madison, New Jersey) by mouth. Beginning 1 hour later, 3 more fluorophotometric scans were taken at intervals of 45 minutes to determine 3 values for the drug-induced reduction of aqueous flow.25 Following each fluorophotometric scan, IOP was measured. These measurements were used to determine outflow facility, the ratio of change in aqueous flow to change in IOP.25-30 Fluorophotometric, rather than tonographic, measurements of outflow facility were used because fluorophotometry avoids pseudofacility and scleral rigidity, factors confounding tonography.30 Uveoscleral outflow was calculated mathematically as the difference between aqueous flow and trabecular outflow.25,30

Eyes from the patients with XFS were chosen to be included in the study (only 1 eye per patient) if they were the only eye to meet the study criteria (usually because of a history of cataract surgery in 1 eye and/or unilateral XFS [n = 31]); if they had greater severity of XFS (based on a higher IOP or more medications [n = 4]); or if they had longer history of XFS (n = 1). Random selection was used in patients with symmetrical XFS (n = 4). For age-matched controls in whom both eyes were eligible for participation, left or right eyes were selected to match the laterality of the corresponding XFS patient’s study eye.

Group means were compared using Mann-Whitney tests with post hoc Bonferroni tests for multiple comparisons, where applicable. Univariate linear regression analyses were used to determine the effects of IOP on various parameters of aqueous humor dynamics when all participants were combined into a single data set. Multivariate linear regression analyses were used to determine if the effects of IOP on aqueous humor dynamics were related to the presence of XFS and vice versa, where XFS was treated as a binary variable. The multivariate linear regression analyses introduced an independent variable interaction term, which is statistically significant in cases in which 1 independent variable affects the dependent variable differently, depending on the value of a second independent variable. Values are reported as mean (SD).

Of the 80 participants enrolled in the study, 28 were male and 52 were female. Two controls were black and all others were white. The postmeasurement analysis used 17 right eyes and 23 left eyes in the XFS groups and 22 right eyes and 18 left eyes in the control groups. The Table includes the IOPs of the XFS and control groups. Those with diagnosed XFS exhibited significantly higher IOPs than their control counterparts (P = .02). The Table and Figure 1 summarize the IOPs of the 4 subgroups. The 2 OHT groups had significantly higher IOPs than their corresponding ocular normotension groups (P < .001 for both comparisons). Additionally, the ocular normotension control group exhibited a significantly lower mean IOP than either XFS group (P = .005 compared with XFS and ocular normotension; P < .001 compared with XFS and XFS). The difference in IOP was not significant for the 2 OHT subgroups.

The episcleral venous pressure was similar in all groups (Table). When all participants in the study were pooled, the presence of XFS did not affect episcleral venous pressure, but a positive correlation existed between IOP and episcleral venous pressure (R2 = 0.13, P = .001). The multivariate linear regression model indicated that the correlation between IOP and episcleral venous pressure (R2 = 0.21, P = .005) was independent of the presence of XFS. The mean aqueous flow rates for each group ranged from 1.96 to 2.34 µL/min, were statistically similar in all groups (Table), and were not correlated with IOP or affected by the presence of XFS according to univariate and multivariate analyses.

The OHT group had a significantly lower mean outflow facility than the ocular normotension group (P = .03) (Table and Figure 2A). All other comparisons of outflow facility between and among groups were not differ-
The combined XFS groups exhibited a significantly lower mean uveoscleral outflow compared with the combined OHT and ocular normotension group (P < .001) (Table and Figure 3B). Uveoscleral outflow was not different when comparing each of the 2 XFS groups with one another or the OHT and ocular normotension groups with one another (Table and Figure 3A). Uveoscleral outflow was lower in those with XFS and OHT compared with OHT alone (P = .02) and in those with XFS and ocular normotension compared with ocular normotension alone (P = .06) (Table and Figure 3A). The inverse relationship between uveoscleral outflow and the presence of XFS was independent of IOP according to a multivariate analysis (R² = 0.21, P = .02), whereas IOP was not correlated with uveoscleral outflow.

Mean anterior chamber volume was 199 (40) µL in all participants with XFS (n = 40) and 169 (42) µL in all controls (n = 40). In the 4 groups, anterior chamber volume averaged 191 (40) µL in those with XFS and ocular normotension (n = 25), 166 (39) µL in those with ocular normotension (n = 25), 212 (38) µL in those with XFS and OHT (n = 15), and 174 (48) µL in those with OHT (n = 15).

Corneal thickness averaged 565 (43) µm in all participants with XFS (n = 40) and 536 (39) µm in all controls (n = 40). In the 4 groups, mean corneal thickness was 574 (48) µm in those with XFS and ocular normotension (n = 25), 548 (40) µm in those with ocular normotension (n = 25), 550 (26) µm in those with XFS and OHT (n = 15), and 516 (27) µm in those with OHT (n = 15).

This is the first study to demonstrate that uveoscleral outflow is reduced in XFS. It is a prospective study that includes appropriate age-matched controls. The uveoscleral outflow effect is independent of IOP and consistent with the pathophysiology of the condition. Exfoliation syndrome is associated with significantly upregulated homocysteine levels in the aqueous humor, upregulated transforming growth factor β1 expression in anterior seg-

### Table. Comparison of Aqueous Humor Dynamics Among Patients With XFS With or Without OHT and Age-Matched Controls With or Without OHT

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Patients With XFS and ONT (n = 25)</th>
<th>Controls With OHT (n = 25)</th>
<th>Patients With XFS and OHT (n = 15)</th>
<th>Controls With OHT (n = 15)</th>
<th>All Patients With XFS (n = 40)</th>
<th>All Controls (n = 40)</th>
<th>P Valuea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>73 (8)</td>
<td>73 (8)</td>
<td>70 (8)</td>
<td>70 (7)</td>
<td>72 (8)</td>
<td>72 (7)</td>
<td>.61</td>
</tr>
<tr>
<td>Intraocular pressure, mm Hg</td>
<td>16.7 (2.4)</td>
<td>14.0 (2.4)</td>
<td>23.4 (6.5)</td>
<td>21.1 (3.4)</td>
<td>19.2 (5.4)</td>
<td>16.7 (4.4)</td>
<td>.02</td>
</tr>
<tr>
<td>Episcleral venous pressure, mm Hg</td>
<td>8.4 (1.2)</td>
<td>8.6 (1.4)</td>
<td>8.9 (1.4)</td>
<td>9.9 (1.1)</td>
<td>8.6 (1.3)</td>
<td>9.1 (1.4)</td>
<td>.14</td>
</tr>
<tr>
<td>Aqueous flow, µL/min</td>
<td>1.96 (0.70)</td>
<td>2.16 (0.61)</td>
<td>2.21 (0.78)</td>
<td>2.34 (0.61)</td>
<td>2.05 (0.73)</td>
<td>2.23 (0.61)</td>
<td>.07</td>
</tr>
<tr>
<td>Outflow facility, µL/min/mm Hg</td>
<td>0.24 (0.13)</td>
<td>0.32 (0.22)</td>
<td>0.19 (0.19)</td>
<td>0.14 (0.10)</td>
<td>0.22 (0.16)</td>
<td>0.25 (0.20)</td>
<td>.74</td>
</tr>
<tr>
<td>Uveoscleral outflow, µL/min</td>
<td>0.12 (0.60)</td>
<td>0.69 (0.93)</td>
<td>0.10 (0.85)</td>
<td>0.93 (0.55)</td>
<td>0.11 (0.69)</td>
<td>0.78 (0.81)</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

Abbreviations: OHT, ocular hypertension; ONT, ocular normotension; XFS, exfoliation syndrome.

*Comparing all patients with XFS with all controls, Mann-Whitney test. Comparisons between the other 4 groups were made by Mann-Whitney tests with Bonferroni corrections for multiple comparisons. (P < .05; †P < .01). The boxes are bound by the 25% and 75% quartiles; the lines through the boxes represent the median; the error bars end at the farthest data points that are not outliers; and the open circles indicate outliers (which are located at greater than 1.5 times the interquartile range either above or below the quartiles). OHT indicates ocular hypertension; ONT, ocular normotension; XFS, exfoliation syndrome.

## Comment

This is the first study to demonstrate that uveoscleral outflow is reduced in XFS. It is a prospective study that includes appropriate age-matched controls. The uveoscleral outflow effect is independent of IOP and consistent with the pathophysiology of the condition. Exfoliation syndrome is associated with significantly upregulated homocysteine levels in the aqueous humor, upregulated transforming growth factor β1 expression in anterior seg-

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**Figure 1.** Intraocular pressure was measured by pneumotonometry. Comparisons between groups were made using Mann-Whitney tests with Bonferroni corrections for multiple comparisons (†P < .05; †P < .01). The boxes are bound by the 25% and 75% quartiles; the lines through the boxes represent the median; the error bars end at the farthest data points that are not outliers; and the open circles indicate outliers (which are located at greater than 1.5 times the interquartile range either above or below the quartiles). OHT indicates ocular hypertension; ONT, ocular normotension; XFS, exfoliation syndrome.
ment tissues, and reduced matrix metalloproteinase activity. All of these factors impair the stability, organization, and integrity of the extracellular matrix. Because the extracellular matrix of the ciliary muscle appears to be an important factor influencing the rate of uveoscleral outflow, it is not surprising that a reduction in the outflow of aqueous humor through this pathway was found in XFS.

We used an indirect method for mathematically calculating uveoscleral outflow based on an expanded version of the Goldmann equation and experimental measurements of aqueous flow, outflow facility, IOP, and episcleral venous pressure. Currently, this is the only method to assess uveoscleral outflow in humans and it is not without its limitations. The calculation assumes that uveoscleral outflow is independent of IOP. Deviations from this assumption may cause errors in calculating uveoscleral outflow. Because uveoscleral outflow is so low in XFS, a difference in episcleral venous pressure
as little as 1 mm Hg could change the calculated uveoscleral outflow value from a positive to a negative number. It is important to emphasize that each of the assumptions in the determination of uveoscleral outflow is made for all participants, and group mean differences reflect differential uveoscleral outflow rates associated with the various ocular conditions. Alternatively, negative uveoscleral outflow values may actually have a true physiological correlate in the form of reverse aqueous humor flow and/or fluorescein diffusion into the anterior chamber from the suprachoroidal space or the iris.

The mechanism of action for prostaglandin analogues, a widely prescribed class of topical ocular hypertensive medications, appears to partially increase uveoscleral outflow via remodeling of the extracellular matrix of the ciliary body. As such, prostaglandin analogues might be especially effective in patients with XFS. Indeed, patients with XFS seem to respond to prostaglandin analogues at least as well as patients with OHT or primary open-angle glaucoma, though enhanced efficacy of these drugs in patients with XFS compared with patients without could be partially attributable to higher pretreatment IOPs, which are generally exhibited by patients with XFS. In patients with XFS, prostaglandin analogues demonstrate greater IOP control than β-blocker monotherapy; prostaglandin analogue/α-2 agonist combination therapy is better than β-blocker/carbonic anhydrase inhibitor fixed combination therapy; and prostaglandin analogue monotherapy is comparable with topical β-blocker/pilocarpine combination treatment and with or only slightly less effective than β-blocker/carbonic anhydrase inhibitor combination therapy. While many of these comparisons also hold for patients with OHT and primary open-angle glaucoma, they demonstrate that prostaglandin therapy is effective at lowering IOP in XFS. However, it should be noted that even with a common IOP, patients with XFS might be at a greater risk for glaucomatous onset and progression than those without XFS. In a study that investigated cases of unilateral XFS with symmetrical IOPs, fluorescein disc changes occurred only in the eyes with XFS. Thus, patients with XFS may require more aggressive ocular hypertensive treatment than patients with primary open-angle glaucoma to maintain a comparable rate of disease progression.

Interestingly, latanoprost has been shown to normalize the levels of various factors found to be at abnormal concentrations in the aqueous humor of XFS eyes, such as those involving the action of matrix metalloproteinases. This may indicate a further advantage beyond the mechanism of IOP reduction of treating XFS patients with prostaglandin analogues.

This study demonstrates that outflow facility tends to be lower in XFS when the condition is accompanied by OHT. This finding is consistent with the observations that increased resistance to outflow is found in eyes with XFS and very high IOPs compared with controls, but no such significant difference is found in eyes with unilateral XFS and normal pressures compared with the fellow eye. The outflow facility reduction in patients with XFS and OHT may be unrelated to their XFS and may be similar to the cause of OHT in patients without XFS. As such, an indirect association between XFS and outflow facility may exist and may be related to IOP as a confounding variable. Outflow facility is lower in eyes with OHT than in normotensive eyes; the fact that OHT often occurs in eyes with XFS might account for the reduction in outflow facility that is often observed in conjunction with XFS. However, evidence opposed to this theory suggests that different mechanisms cause a reduction in outflow facility with or without XFS. Juxtacanicular plaque and trabecular cell loss, which may contribute to a decreased outflow facility in patients with glaucoma, have not been reported in XFS. However, exfoliative material in the trabecular meshwork has been discovered. As an alternative explanation, XFS may cause a reduction in outflow facility but only in a certain subset of patients with XFS who go on to develop OHT. Therefore, exfoliative material could contribute to impairment in conventional outflow, and, with increasing severity, would lead to OHT by reducing outflow facility.

Subtle, statistically insignificant trends in the current data might support this latter theory. Our study demonstrated a rather high level of variability in outflow facility measurements made in healthy controls (Figure 2A). It is possible that this reflects the fact that healthy controls with normal IOPs can sometimes have very high outflow facilities, even approaching 0.8 µL/min/mm Hg (Figure 2A). The other 3 groups in our study exhibited lower and more limited ranges of outflow facility values, possibly because each of the members of these other groups had at least 1 condition associated with impaired aqueous outflow, ie, OHT and/or XFS. Specifically, this difference between the 2 ocular normotension groups might reflect a tendency toward impairment in outflow facility that is associated with XFS but only becomes statistically significant as IOP rises. Alternatively, the difference in variability between groups may simply be due to measurement error and/or the presence of outliers. Fluorophotometric measurements of outflow facility are more difficult in patients with lower baseline IOPs, as aqueous flow suppressants tend to have a smaller effect on IOP.

We found no association between aqueous flow and either IOP or the presence of XFS. An association between IOP and episcleral venous pressure was found. This finding is similar to a recently published study, which found a positive correlation between IOP and episcleral venous pressure in patients with primary open-angle glaucoma and normal-tension glaucoma. We did not find any association between episcleral venous pressure and the presence of XFS. In this study, episcleral venous pressure was measured once at mid-morning before the administration of aqueous flow suppressors. Importantly, previous studies have demonstrated that neither topical β-blockers nor carbonic anhydrase inhibitors significantly alter episcleral venous pressure and that episcleral venous pressure exhibits minimal diurnal variation. As such, our study’s single episcleral venous pressure measurement was sufficient to accurately calculate conventional outflow and uveoscleral outflow rates.

Apparent similarities exist between the pathophysiology of pigment dispersion syndrome and XFS, ie, accumulation of abnormal material at the site of conven-
tional drainage and reduction in outflow facility.\textsuperscript{22,64,65} Pigment dispersion syndrome is a known cause of glaucoma that has been linked to elevated IOPs.\textsuperscript{60,67} Studies of aqueous humor dynamics in patients with pigment dispersion syndrome report a reduction in outflow facility\textsuperscript{64,65} but no change in uveoscleral outflow.\textsuperscript{65} The etiology of this finding is likely due to an accumulation of pigment in the trabecular meshwork and a subsequent degradation of the conventional outflow pathway. Gotta et al\textsuperscript{68} found that pigment granules within the trabecular meshwork cells of eyes with pigment dispersion syndrome were positively associated with decreased trabecular cell numbers, collapse of the intratrabecular spaces, and an increase in extracellular material in the meshwork, all of which probably contribute to impaired conventional outflow and development of OHT. The release of pigment in pigment dispersion syndrome does not appear to affect the uveoscleral pathway,\textsuperscript{65} which apparently is less susceptible to pigment accumulation.

In contrast to pigment dispersion syndrome, XFS might involve changes in the ciliary muscle and the surrounding extracellular matrix, including ciliary epithelial cell degeneration caused by exfoliative material--induced basement membrane destruction.\textsuperscript{68} Furthermore, XFS is a disorder of the extracellular matrix that results in increased matrix accumulation possibly due to alterations in matrix metalloproteinase concentrations.\textsuperscript{35} Uveoscleral outflow appears to be affected by the microarchitecture of the ciliary muscle extracellular matrix.\textsuperscript{70} Consistent with this observation, our study demonstrates that uveoscleral outflow is reduced in patients with XFS. Therefore, the mechanism of OHT in XFS appears to be different from that in pigment dispersion syndrome.

We demonstrate that XFS is associated with a reduction in aqueous outflow through the uveoscleral pathway. Patients with XFS and OHT also exhibit a decrease in outflow facility.\textsuperscript{22} Therefore, XFS patients with high IOPs are likely to demonstrate significant impairments in both aqueous humor outflow pathways. These findings of reduced uveoscleral outflow may explain the higher IOPs that are difficult to control, which in turn contribute to an enhanced risk of glaucomatous optic neuropathy. By targeting the specific changes in the eyes of patients with XFS, improved therapeutic modalities may be developed specifically for this condition.

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\textbf{REFERENCES}
