Aqueous humour dynamics in uveitic eyes

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Abstract

Purpose: To investigate aqueous humor dynamics in uveitic eyes

Design: A cross-sectional study

Participants: Patients with recurrent (≥3 attacks) anterior uveitis (now quiescent) and being treated for glaucoma or OHT (group-1), previous recurrent anterior uveitis (≥3 attacks) without glaucoma or OHT (group-2), and normal subjects with no ocular problems and IOP < 21 mmHg at screening, formed the control group (group-3).

Methods: Patients had one-off measurements. Group-1 patients who were on anti-hypertensives, were washed out for a 4-week period, prior to their study measurements.

Main outcome measure: Tonographic outflow facility, aqueous flow rate and uveoscleral outflow.

Results: One hundred and one patients were screened between February 2014 and February 2017. Nine patients did not meet the inclusion criteria. Groups-1 and-3 each included 30 patients, and group-2 included 32 patients.

The mean IOP was higher in the group-1 compared to the others (25±10.2 (group-1) vs 16±2.7 (group-2) vs 16±2.2 mmHg (group-3), p < 0.001).

The tonographic outflow facility was lower in group-1 compared to the others (0.18±0.1 (group-1) vs 0.25±0.1 (group-2) vs 0.27±0.1 µl/min/mmHg (group-3), p = 0.005). However, aqueous flow rate was not statistically different (2.47±0.9 (group-1) vs 2.13±0.9 (group-2) vs 2.25±0.7 µl/min (group-3), p = 0.3). There was also no significant difference in calculated uveoscleral outflow.

Conclusion: This is the first aqueous humor dynamic study in patients with uveitic glaucoma/OHT and recurrent anterior uveitis compared with age-matched controls. We have demonstrated that the elevated IOP seen in the uveitic glaucoma/OHT
eyes (3-6 attacks), was due to reduced tonographic outflow facility. The aqueous humor flow rate was not detectibly different nor did the calculated uveoscleral outflow demonstrated any discernible difference. However, the exact mechanism remains to be elucidated.
Aqueous humour dynamics in uveitic eyes

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Introduction

Uveitis is one of the most common ophthalmic condition which is seen in eye departments globally. The prevalence of uveitis varies in different parts of the world\(^1\)-\(^3\), however, idiopathic recurrent anterior uveitis\(^4,5\) is the most common diagnosis in those affected. One of the most serious sight threatening sequelae from this condition is uveitic glaucoma, which has a reported incidence of between 5 to 24\(^6\)-\(^8\) amongst long-term uveitic eyes. Despite the relatively high prevalence, there have only been a few previous aqueous humour dynamic studies exploring the pathogenesis of uveitic glaucoma in humans. This is partly due to measurement challenges which are unfortunately quite common in uveitic glaucoma/OHT cases. Firstly, eyes with prior intraocular surgery, such as cataract surgery, glaucoma filtration surgery or iridotomy/iridectomy, must be excluded. This is due to irido-lenticular barrier compromise which subsequently may lead to excessive posterior flow of fluorescein and thereby disrupt the fluorescein loss assumptions underlying the fluorophotometry measurements\(^9,10\). Secondly, active uveitis can render the fluorophotometry measurements inaccurate. This is because of the presence of excessive protein in the anterior chamber which can bind with the fluorescein molecule and disrupt its natural clearance from the anterior chamber\(^9,10\). Considering these limitations, it is not surprising that there have only been two previous human aqueous humour dynamics studies in uveitis or uveitic glaucoma/OHT and both were in those with active uveitis. The first study was by Ladas and associates\(^11\) who only investigated the correlation between tonographic outflow facility measured by Schiøtz tonography and laser flare photometry. However, they did not measure the aqueous flow rate. Their cohort comprised of patients with active uveitis (any type of uveitis including pan-uveitis and posterior uveitis were included). None of the eyes had any history of raised intra-ocular pressure (IOP). They showed that higher flare measurement in the anterior chamber coincided with lower outflow facility.

The other study by Johnson et al.\(^12\), compared tonographic outflow facility (Schiøtz) and aqueous flow rate (fluorophotometry) in 10 eyes with cyclitis to their fellow healthy eyes. The results showed that the aqueous flow rate and facility of outflow were not significantly different compared to unaffected fellow eyes. Only three cases had raised IOP at the time of the measurement. They also had some anterior chamber activity (trace to 2+ cells and flare in the anterior chamber was reported) which may have compromised the accuracy of the tonographic outflow facility and aqueous flow rate measurements.

In regard to aqueous humour dynamics, there are probably three distinct circumstances where uveitis can lead to raised intra-ocular pressure; (1) those eyes that are currently actively inflamed, with anterior chamber inflammatory cells and proteins as well as concurrent steroid use; (2) previous severe or multiple uveitis attacks with a high likelihood of having had incisional ocular surgeries such as glaucoma filtration or cataract surgeries (such as juvenile chronic arthritis associated uveitis); (3) previous multiple but moderate anterior uveitis attacks but currently quiescent.

As fluorophotometry can be inaccurate in eyes that are actively inflamed, we have therefore designed this study specifically investigating quiescent eyes of the last category of the aforementioned list. This helps us understand why some eyes develop uveitic glaucoma/OHT after only a few attacks of anterior uveitis and not others.
Materials and methods

This study is a cross-sectional study comparing aqueous humour dynamics in age-matched healthy volunteers, previous anterior uveitis (without glaucoma) and those with uveitic glaucoma/OHT but no active uveitis at the time of study measurement. Ethical approval for this study was obtained from the local St Thomas’ Hospital research ethics committee. This research followed the tenets of the Declaration of Helsinki (http://www.clinical-trials.gov, identifier NCT02765308, May 6, 2016). Healthy volunteers were recruited from the hospital staff and their family members after having a comprehensive ophthalmic examination. Patients with recurrent anterior uveitis were identified from our uveitis and glaucoma clinics at St Thomas’ Hospital, London, United Kingdom. A patient information leaflet was provided at the initial contact, and signed informed consent was obtained before glaucoma eye drop washout and study measurements took place. Participants enrolled in the study were divided into three groups (1) previous recurrent anterior uveitis (≥ 3 attacks) with glaucoma/OHT (uveitic glaucoma) on topical glaucoma drops treatment (group 1); (2) previous recurrent anterior uveitis (≥ 3 attacks) with normal IOP and no diagnosis of glaucoma (group 2); and (3) healthy volunteers (group 3). All uveitic eyes were quiescent at the time of enrolment (defined by absence of any flare/cells on slit lamp biomicroscopy). All patients in the uveitic glaucoma/OHT group had a 4-week washout period from their anti-hypertensive and steroid treatments prior to their study measurements (patients had a safety visit two-weeks after commencing the washout).
**Eligibility criteria**

**Inclusion Criteria**
- Age >18 years
- Adequate cognitive function and ability to understand verbal and written information in English
- Previous recurrent anterior uveitis (≥ 3 attacks) with/without OHT/glaucoma

**Exclusion Criteria**
- Other glaucoma diagnosis including pigment dispersion syndrome and pseudoexfoliation
- Active uveitis
- Ocular trauma
- Intraocular or keratorefractive surgery
- Use of systemic medication that may affect aqueous humour production such as beta-blockers
- A history of allergy or hypersensitivity to fluorescein
- Any abnormalities preventing reliable IOP or fluorophotometric readings

**Primary outcome measures**

- Facility of tonographic outflow (measured by digital Schiøtz tonometry)
- Aqueous flow rate (measured by fluorophotometry)
- Uveoscleral outflow (calculated from the Goldmann’s equation)

**Measurements**

All patients underwent clinical ophthalmic examinations including visual acuity, slit lamp biomicroscopy, gonioscopy, anterior chamber depth, and axial length measurement (IOL Master; Carl Zeiss Meditec Inc., Dublin, CA), central corneal thickness (CCT; Pachmate DGH 55, DGH Technology, Inc., Exton, PA), visual fields (Humphrey automated white-on-white, 24-2 SITA-standard; Carl Zeiss Meditec), and dilated ophthalmoscopy. The night before (10 PM) the fluorophotometric scans, participants self-administered from 3 to 6 drops of fluorescein sodium 2% (Minims; Bausch & Lomb, Kingston-upon-Thames, UK) topically into both eyes at 5-minute intervals depending on their ages (age < 26 years, 5 to 6 drops; age 26–35 years, 4 drops; >35 years of age, 3 drops). Fluorophotometry was performed in both eyes with a scanning ocular fluorophotometer (FM-2, Fluorotron Master ocular fluorophotometer; OcuMetrics, Mountain View, CA) from 9 AM to 12 noon. The aqueous flow rate was determined using dedicated software provided with the fluorophotometer. Duplicate or triplicate scans were collected and repeated at 1-hour intervals for four measurements to determine the aqueous flow rate (Ff). Following each set of scans, IOP was measured using pneumotonometry (Model 30 Classic; Reichert Ophthalmic Instruments, Depew, NY); IOP was recorded as the arithmetic mean of a total of 12 measurements per eye (3 measurements every hour alternating between eyes).

Tonographic outflow facility (C) was measured by constant weight tonography (5.5, 7.5 or 10 g) using a modified digital Schiøtz tonographer (designed by the Department of Bioengineering, Imperial College, London, UK) at 10 – 11 AM. Our device used an original Schiøtz tonographer footplate from a commercially available unit (model 720, Berkeley Bioengineering Inc., San Leandro, CA, USA) attached to a
3D printed shell that was designed such that the weight conformed to the specifications set out by the Committee on Standardization of Tonometers\textsuperscript{14}. Displacement of the weighted plunger was measured using a linear variable differential transformer (LVDT; MHR, TE Connectivity, Schaffhausen, CH, USA) driven by a signal conditioner (AD698, Analog Devices, Norwood, MA, USA) and captured digitally by a data acquisition system (USB-6009, National Instruments, Austen, TX, USA). Validation studies confirmed that the LVDT voltage output was linear with respect to the Schiøtz scale reading (Supplemental Figure 1), where each scale reading is equivalent to 0.05 mm of plunger displacement\textsuperscript{14}. Facility was estimated using Grant’s equation (Equation 1)\textsuperscript{15}.

\[
C = \frac{V_{c,t} - V_{c,0} + \frac{1}{K} (\log P_{t,0} - \log P_{t,t})}{\left(\frac{P_{t,0} + P_{t,t}}{2} - P_0 - \Delta P_v\right) t}
\]

Equation 1

where \(V_{c,t}\) and \(V_{c,0}\) are the aqueous volumes displaced at time \(t\) and at the start of tonography \((t = 0)\). \(P_{t,t}\) and \(P_{t,0}\) are values of IOP at time \(t\) and at the start of tonography. \(P_0\) is the IOP immediately prior to the start of tonography. \(\Delta P_v\) is the change in episcleral venous pressure, assumed to be 1.25 mmHg\textsuperscript{16}, and \(K\) is the coefficient of ocular rigidity, assumed to be 0.0215/\mu l\textsuperscript{-1}\textsuperscript{17}. \(V_{c,t}, V_{c,0}, P_{t,t}, P_{t,0}\) and \(P_0\) were determined based on the value of the Schiøtz scale reading and tables provided by Moses and Becker\textsuperscript{18}. By minimising the root mean square error between the tonographic tracing and Equation 1 (Supplemental Figure 2), the optimal value of \(C\) was determined numerically.

At present the clinical measurement of uveoscleral outflow in humans is not possible\textsuperscript{19}; hence this value is generally calculated from the Goldmann’s equation. Sit and McLaren used a computerized venomanometry to measure episcleral venous pressure (EVP)\textsuperscript{20, 36}. They illustrated that EVP in normal subjects can vary between 6 and 10 mmHg. Therefore, we have used this EVP range for our calculations. However, to make this calculation valid, it must be assumed that the episcleral venous pressure did not vary significantly between all three groups of patients in our study.
Uveoscleral outflow was calculated using Goldmann’s equation (Equation 2) with an assumed episcleral venous pressure of 6-10 mmHg. “\(Ff\)” is the rate of aqueous humor formation measured by fluorophotometry, “\(C\)” is the tonographic facility of outflow, “\(Pi\)” is the intraocular pressure, “\(Pe\)” is the episcleral venous pressure, and “\(Fu\)” is uveoscleral flow. 

\[
Ff = (Pi - Pe)C + Fu \quad \text{Equation 2}
\]

Therefore,

\[
Fu = Ff - C(Pi - Pe) \quad \text{Equation 3}
\]

Only one randomly (Excel random number generator; Microsoft, Redmond, WA) chosen eye per participant was included in the data analysis, when both eyes fulfilled the inclusion criteria.

**Sample size calculation**

The sample size estimate was based on the results of paired measurements of two parameters (aqueous flow and facility of outflow) in a previous study done at the Mayo clinic, Rochester, MN, USA by the chief investigator (KSL)\(^{21}\). This study had a 90% chance of finding a 5% difference in IOP, 5.4% difference in aqueous flow, and 7.5% difference in outflow facility among medication groups, if these differences existed (\(n=30\) subjects, \(\alpha = 0.05\), and \(\beta = 0.10\)).

**Data analysis**

Histograms and Shapiro-Wilk test were performed to test for normality of distribution of data. A Shapiro-Wilk \(W > 0.05\) was evidence of normal distribution. Student’s t-test was used to compare continuous variables among groups. When data did not follow normality, non-parametric methods of analysis (Mann-Whitney U and Kruskal-Wallis tests) were used. Linear regression analyses were used to determine the correlation of one parameter versus another parameter of aqueous humor dynamics. P 0.05 was considered statistically significant (all analyses, SPSS 24.0; SPSS, Chicago, IL).
Results

We screened one hundred and one patients between February 2014 and February 2017. Nine patients did not meet the inclusion criteria. Thirty patients with recurrent anterior uveitis and being treated for secondary glaucoma/OHT (group 1) and 32-patients with previous recurrent anterior uveitis (group 2) without OHT or glaucoma and with normal IOP who met the inclusion/exclusion criteria were recruited. Thirty healthy volunteers were enrolled as controls (group 3) over the same period. There was a female preponderance in groups 2 and 3. Most subjects were white Caucasians with a few black African/Caribbean and Asians in groups 2 and 3 but slightly higher presence of black patients in group 1 (p=0.06). The mean age in all 3 groups were comparable (p=0.3). The best corrected visual acuity in the uveitic glaucoma/OHT (group 1) was worse compared to other groups (p=0.002). This was primarily due to lens opacity and severity of glaucoma in the group 1. Anterior chamber depth, axial length and central corneal thickness were similar across all groups (p=0.3). Visual field parameters were worse in group 1 compared to other two groups (p=0.05). All patients had open angles on gonioscopy with only five (17%) patients in group 1 with non-contiguous patchy peripheral anterior synchiae. The subjects’ baseline characteristics are summarised in Table 1. Mean intraocular pressure was significantly higher in the uveitic glaucoma/OHT group compared to the other two groups (p<0.001). The tonographic outflow facility (C) was markedly lower in the uveitic glaucoma/OHT group compared to the other two groups (p=0.005). However, aqueous flow rate was not statistically different between the three groups (2.46±0.9 (group 1) vs 2.13±0.9 (group 2) vs 2.25±0.7 (group 3) µl/min, p=0.3). Additionally, no significant difference in uveoscleral outflow (with assumed episcleral venous pressure of 6-10 mmHg based on Sit and McLaren’s work20) was detected between the three groups, even when allowing episcleral venous pressure to vary over the range of 6-10 mmHg (but assuming a uniform pressure for all patients) (p=0.9). The full aqueous humour dynamic results are shown in Table 2.

A break-down of different uveitis diagnoses in groups 1 and 2 are provided in Table 3. Most cases were idiopathic anterior uveitis.

The study eyes in the group 1 on average had 4.5 episodes (range 3-6) of uveitis attacks whilst eyes in the group 2 had on average 6 episodes (range 3-10) prior to study measurements.

If, rather than including all individuals in the uveitic glaucoma/OHT group as in Table 2, we excluded 14 individuals who maintained a normal IOP (< 21 mmHg) post washout, the average IOP in the uveitic glaucoma/OHT group increased to 31.8±9.7 mmHg, which was greater than either the uveitis group 2 (16±2.5 mmHg) and control group 3 (16±2.2 mmHg; p<0.001). Correspondingly, the tonographic outflow facility in the uveitic glaucoma/OHT group excluding those with normal IOP post-washout was 0.13±0.1 µl/min/mmHg, which was significantly lower than either the uveitis group 2 (0.27±0.1 µl/min/mmHg) or control group 3 (0.25±0.1 µl/min/mmHg; p<0.001). Nonetheless, even after removing those with normal post-washout IOP, there was still no statistically significant difference in aqueous flow rate between three groups (2.42±0.9 (group 1) vs 2.18±0.9 (group 2) vs 2.32±0.8 µl/min (group 3), p=0.4). Similarly, the uveoscleral outflow was comparable between all groups (-0.01±2.06 vs 0.64±1.3 in uveitis (group 2) vs 0.75±1.4 in controls (group 3), p=0.1).
Correlation was made between IOP and either aqueous flow rate, tonographic outflow facility, uveoscleral outflow, ACD or AXL. Only the correlation between post washout IOP and tonographic outflow facility was statistically significant with a strong negative correlation observed in group 1 ($R^2=0.86$, $p<0.001$). The other correlations were not significant ($p>0.09$) (Figure 1). However, correlation between IOP and aqueous humour parameters was not significant in other groups ($P=0.6$).
Discussion

This is the first aqueous humour dynamic study in patients with uveitic glaucoma/OHT and recurrent anterior uveitis compared with age-matched healthy controls. However, one should be mindful of the fact that uveoscleral outflow was calculated by the Goldmann formula using assumed episcleral venous pressure. Additionally, we utilised indirect techniques to measure the aqueous flow rate and tonographic outflow facility. Consequently, these parameters may have been compromised by subclinical inflammation. The uveitis and uveitic glaucoma/OHT cases were all clinically quiescent at the time of enrolment and none in the uveitic glaucoma/OHT group had more than six previous anterior uveitis attacks. We demonstrated that the elevated intraocular pressure seen in the uveitic glaucoma/OHT eyes was due to reduced tonographic outflow facility (this is used as a proxy of measuring trabecular outflow facility). The aqueous flow rate was not detectibly different amongst the 3 groups nor did the calculated uveoscleral outflow demonstrated any difference between three groups.

This study is unique in several aspects. Firstly, it encompasses age-matched healthy controls as well as those with previous recurrent anterior uveitis with or without glaucoma/OHT. Additionally, in this study, previously treated uveitic glaucoma/OHT patients underwent a 4-week washout period from their glaucoma medications, mydriatics and steroids before the study measurements. It is therefore suggestive that the reason for raised intraocular pressure, after moderate number of recurrent anterior uveitis attacks, is caused by increased tonographic outflow resistance without significant impairment to aqueous production.

Although the reduction in tonographic outflow facility in our finding should not come as a surprise (as in almost all other types of glaucoma, tonographic outflow impairment is the primary cause of raised IOP), this is the first study to confirm this in uveitic glaucoma. Ladas and associates investigated the correlation between outflow facility measured by Schiøtz tonography and laser flare photometry in patients with active uveitis. They demonstrated that the higher the measured flare in the anterior chamber (>20 photon units/msec), the lower the outflow facility (0.21±0.12 µl/min/mmHg). They also reported that patients with flare <20 photon units/msec had a similar outflow facility to normal controls. Although increased aqueous protein level may lead to obstruction of trabecular meshwork pores in the acute phase, it may also have lasting effect on outflow facility as demonstrated by Epstein et al. They explored the facility of outflow in enucleated human eyes by infusing the eyes with human plasma and showed that facility of outflow reduced by over 40% and interestingly this was not resolved by irrigating the eyes with balanced salt solution. The authors speculated that this may be due to adhesion of serum components of plasma to the aqueous outflow system. All uveitic glaucoma/OHT eyes in our cohort had quiescent anterior chamber on slit lamp examination (although they may had subclinical inflammation) at the time of measurements; however, there might have been lasting damage to TM due to repeated anterior chamber inflammation or even previous long-term use of topical steroids which can eventually cause compromised outflow and consequently raised IOP. Mechanical obstruction due to peripheral anterior synechiae could also account for some of the reduced outflow facility. The evidence from animal studies suggests that inflammatory cells can cause blockage of outflow facility by simply clogging the
trabecular meshwork pores\textsuperscript{25}. Chronic inflammation of the trabecular meshwork may lead to scar formation and permanent damage to the underlying tissue\textsuperscript{26}. In a recent multicentre study of risk factors of ocular hypertension in non-infectious uveitis\textsuperscript{6}, the presence of peripheral anterior synechiae (PAS) carried a three-fold risk of developing OHT. Whilst in our study only 17\% of uveitic glaucoma/OHT cases had some degree of non-contiguous PAS and none had more than 180 degrees of PAS, suggesting that raised IOP might have been due to micro-structural damage to the trabecular meshwork. Additionally, extracellular matrix accumulation in the trabecular meshwork or increased continuity of the endothelial basement membrane along Schlemm’s canal coinciding with long-term use of steroids may play a part in obstruction of trabecular outflow and subsequently raised intraocular pressure\textsuperscript{27, 28}.

Calculated uveoscleral outflow in our present study in human uveitic glaucoma/OHT (as a non-invasive direct clinical measurement remains elusive) is in marked contrast to uveoscleral outflow measured in monkeys’ eyes with active uveitis by Toris and Pederson\textsuperscript{29}. In their study using cynomolgus monkeys, the anterior uveitis was artificially induced with intra-cameral injection of albumin. The aqueous flow rate and uveoscleral outflow was measured using fluorescein isothiocyanate (FITC) dextran 70. They found that the uveoscleral outflow was four times greater in the inflamed eyes than the controlled eyes. It is therefore likely that in actively inflamed eyes, with the oedematous ciliary body and supra-choroidal space seen in the monkeys’ eyes, as well as the release of endogenous prostaglandins\textsuperscript{30}, there will be a transient increase in uveoscleral outflow which subsided once the inflammation had settled as in our study. Based on this observation, the authors would like to speculate that topical prostaglandin is more likely to be effective in lowering IOP in quiescent eye than in active uveitis eye.

Post-operative hypotony after glaucoma filtration surgery in uveitic glaucoma has been routinely attributed to ‘aqueous shutdown’ without any evidence to substantiate this claim\textsuperscript{31-33}. Therefore, one of the most interesting findings in our study is the similar level of aqueous production rate in all three groups. This suggests that after less than seven attacks of anterior uveitis, there may not be any significant damage to the ciliary epithelium and other associated apparatus involved in the production of aqueous in human eyes. We limited our uveitis groups to only those eyes with more than three attacks of anterior uveitis and interestingly, none of our recruited cases in group 1 had more than six attacks of uveitis. We identified many other cases of uveitic glaucoma with more than 6 previous attacks, but none were eligible for our study due to previous intraocular surgeries, such as cataract and glaucoma surgeries. It is therefore plausible that uveitic glaucoma patients who have more than six attacks of uveitis may have different aqueous dynamic parameters, including aqueous production rate change.

Based on available evidence and our own study, aqueous production rate is probably only reduced in those cases of severe acute uveitis or those eyes with previous multiple (more than 6) and severe uveitis attacks, such as those associated with idiopathic juvenile arthritis. Therefore, surgical techniques\textsuperscript{34}, rather than ‘aqueous shutdown’ are the most likely cause of hypotony post-glaucoma filtration surgeries, in those glaucoma eyes with moderate uveitis.

As part of the wash-out process before the aqueous humour dynamic measurements, we also observed an interesting finding in our study which may have
significant clinical ramifications. After washout, the mean IOP in nearly half (47%) of the uveitic glaucoma/OHT group was less than 22 mmHg. It is likely that following a period of inactivity of the uveitis as well as cessation of topical steroid treatment, in those cases of presumed uveitic glaucoma/OHT, IOP may revert to normal after wash-out. Clinically, it is therefore sensible to consider treatment washout in this group of patients after these eyes have been controlled on glaucoma drops treatment after 6-12 months without any recurrence of their uveitis.

There are, however, a few inherent limitations in aqueous humour dynamics studies. The most important of all is that the eyes undergoing aqueous humor dynamic measurements should not have had any intraocular surgery such as cataract surgery or iridotomy, which may compromise the irido-lenticular barrier to the posterior flow of fluorescein during fluorophotometry measurement9,10. Active uveitis also renders the fluorophotometry measurement inaccurate due to the presence of excessive protein in the anterior chamber which can bind to fluorescein molecules. Furthermore, with the breakdown of the blood-aqueous barrier, fluorescein can diffuse through unconventional pathways, potentially distorting the assumptions about the standard diffusional loss of fluorescein during fluorophotometry9,10. Tonographic outflow facility is influenced by ‘pseudofacility’ especially in uveitis due to possible subclinical inflammation22. Therefore, there might be a discordance between tonographic outflow facility and trabecular outflow facility. Another issue relates to the measurement of episcleral venous pressure and uveoscleral outflow. At present the precise measurement of uveoscleral outflow is not possible in humans19; hence this value is generally calculated from the Goldmann’s equation. As we are also unable to accurately measure the episcleral venous pressure (EVP)21,35 despite some experimental methods of measuring EVP20, they are not widely available. Therefore, it is generally accepted that EVP to be approximately 10 mmHg in man. To make this calculation valid, it must be assumed that the EVP in these three groups of patients did not vary significantly. We did not perform flare measurement in our patients as we did not have the flare meter in our department; however, as we have taken great care in excluding any eyes with active uveitis, we do not believe that this measurement will affect the main findings of our study.

In summary, to our knowledge this is the first aqueous humor dynamic study in patients with previous recurrent anterior uveitis and uveitic glaucoma/OHT compared with age-matched healthy controls. We have demonstrated that elevated intraocular pressure seen in the uveitic glaucoma/OHT eyes (after less than seven previous attacks of uveitis) was due to reduced tonographic outflow facility alone. The aqueous humour flow rate was not detectibly different among the three groups nor did the calculated uveoscleral outflow demonstrate any detectible difference between the three groups. Clinicians should also consider treatment washout for those with medically treated uveitic glaucoma in the future. However, future studies should be undertaken once we have better techniques of aqueous dynamics measurements in eyes with active uveitis and previous intraocular surgeries as well as non-invasive and accurate techniques for measuring EVP and uveoscleral outflow.
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References
Captions references:
Table 1. Baseline characteristics of all participants are shown.
Table 2. Aqueous humour parameters and comparison made between all 3 groups
Table 3. The uveitis diagnosis in groups 1 and 2 are shown here.

Figure 1. Intraocular pressure (IOP) vs tonographic outflow facility plot. Correlation was made between post washout IOP and tonographic outflow facility.

Supplemental Figure 1
Displacement calibration of the digital Schiotz tonographer. Using a micrometre, the plunger of the LVDT was moved in increments of 50 µm, equivalent to 1 Schiotz scale reading, over the range of -1 to 20 scale readings, whilst measuring the LVDT voltage output (supplemental Figure 1). Each position was measured 2 or 3 times, with all data shown. The voltage-displacement relationship was linear over the full range ($R^2 = 0.9997$), allowing the measured LVDT voltage to be converted into a Schiotz scale reading. A scale reading of -1 is defined as the reading when the footplate is placed on a rigid spherical surface with a 15 mm radius of curvature.

Supplemental Figure 2
A sample tonography tracing fit to Equation 1. The black trace shows the captured signal from the LVDT, converted into Schiotz scale reading (see Supplemental Figure 1). The red curve shows the predicted scale reading based on fitting Equation 1 to the black tracing for the optimal value of $C$ (in this case 0.31 µl/min/mmHg). Tonography was performed with a 5.5-gram weight placed on the right eye of a uveitic patient without or ocular hypertension (IOP = 16.4 mmHg) glaucoma (Figure 2). The high frequency oscillations (~1 Hz) observed in the black tracing reflects the ocular pulse.
Table 1. Baseline characteristics of all participants are shown.

<table>
<thead>
<tr>
<th></th>
<th>Group 1 [uveitic glaucoma/ OHT] (n=30)</th>
<th>Group 2 [uveitis] (n=32)</th>
<th>Group 3 [Normal] (n=30)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Comparison between Group 1 and Group 3</strong></td>
<td>P value</td>
<td>95% CI</td>
<td>P value</td>
</tr>
<tr>
<td><strong>Gender</strong> (F: M)</td>
<td>14(47%): 16(53%)</td>
<td>24(80%): 8(20%)</td>
<td>20(67%): 10 (33%)</td>
</tr>
<tr>
<td></td>
<td>0.06</td>
<td>---</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td>0.01</td>
<td>---</td>
<td>0.01</td>
</tr>
<tr>
<td><strong>Age, years</strong></td>
<td>50±14.0</td>
<td>49±11.1</td>
<td>44±13.6</td>
</tr>
<tr>
<td></td>
<td>0.9</td>
<td>-7.2- 8.4</td>
<td>0.4</td>
</tr>
<tr>
<td><strong>BCVA, LogMAR</strong></td>
<td>0.04±0.2</td>
<td>0.02*</td>
<td>0.002*</td>
</tr>
<tr>
<td></td>
<td>-0.2- -0.01</td>
<td>-0.06±0.1</td>
<td>0.02</td>
</tr>
<tr>
<td><strong>CCT, µm</strong></td>
<td>552±37.0</td>
<td>544±31.2</td>
<td>546±128.2</td>
</tr>
<tr>
<td></td>
<td>0.6</td>
<td>-27.5-11.6</td>
<td>0.7</td>
</tr>
<tr>
<td><strong>ACD, mm</strong></td>
<td>3.34±0.3</td>
<td>3.26±0.4</td>
<td>3.49±0.3</td>
</tr>
<tr>
<td></td>
<td>0.6</td>
<td>-0.3- 0.12</td>
<td>0.3</td>
</tr>
<tr>
<td><strong>AXL, mm</strong></td>
<td>23.4±4.0</td>
<td>23.8±1.4</td>
<td>24.2±1.5</td>
</tr>
<tr>
<td></td>
<td>0.8</td>
<td>-1.2- 1.9</td>
<td>0.5</td>
</tr>
<tr>
<td><strong>MD, dB</strong></td>
<td>-3.79±6.1</td>
<td>-1.80±2.4</td>
<td>-1.36±1.8</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>-0.3- 4.3</td>
<td>0.05*</td>
</tr>
</tbody>
</table>

Table 2. Aqueous humour parameters and comparison made between all 3 groups

<table>
<thead>
<tr>
<th></th>
<th>Group 1 [Uveitic glaucoma/OHT] (n=30)</th>
<th>P value</th>
<th>95% CI</th>
<th>Group 2 [Uveitis] (n=32)</th>
<th>P value</th>
<th>95% CI</th>
<th>Group 3 [Normal] (n=30)</th>
<th>P value</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>IOP, mmHg (min-max)</td>
<td>25±10.2 (10-52)</td>
<td><em>&lt;0.001</em></td>
<td>-12.5-4.6</td>
<td>16±2.5 (10-22)</td>
<td><em>&lt;0.01</em></td>
<td>4.3-12.5</td>
<td>16±2.2 (11-19)</td>
<td>0.9</td>
<td>-3.6-4.7</td>
</tr>
<tr>
<td>Aqueous flow rate (Ft), µl/min</td>
<td>2.47±0.9</td>
<td>0.3</td>
<td>-0.8-0.21</td>
<td>2.18±0.9</td>
<td>0.7</td>
<td>-0.66-0.37</td>
<td>2.32±0.8</td>
<td>0.7</td>
<td>-0.36-0.65</td>
</tr>
<tr>
<td>Trabecular outflow facility (C), µl/min-mmHg</td>
<td>0.18±0.1</td>
<td><em>0.005</em></td>
<td>0.02-0.15</td>
<td>0.27±0.1</td>
<td><em>0.04</em></td>
<td>0.01-0.14</td>
<td>0.25±0.1</td>
<td>0.7</td>
<td>-0.08-0.04</td>
</tr>
<tr>
<td>Uveoscleral outflow (Fu), 10, mmHg</td>
<td>0.49±1.6</td>
<td>0.9</td>
<td>-0.73-1.02</td>
<td>0.64±1.3</td>
<td>0.7</td>
<td>-0.62-1.15</td>
<td>0.75±1.4</td>
<td>0.9</td>
<td>-0.75-0.99</td>
</tr>
</tbody>
</table>

IOP: intraocular pressure, OHT: ocular hypertension. FU: calculated uveoscleral outflow assumed
Episceral venous pressure of 10 mmHg
Table 3. The uveitis diagnosis in groups 1 and 2 are shown here.

<table>
<thead>
<tr>
<th></th>
<th>Idiopathic</th>
<th>Sarcoidosis</th>
<th>HLA-B27 associated</th>
<th>Psoriasis</th>
<th>Tuberculosis related</th>
<th>Herpetic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 (n=30)</td>
<td>21</td>
<td>4</td>
<td>3</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Group 2 (n=32)</td>
<td>22</td>
<td>2</td>
<td>7</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
IOP vs tonographic outflow facility plot

- Patients
  - Group 1
  - Group 2
  - Group 3

Mean post washout IOP, mmHg

Mean tonographic outflow facility flow, μl/min/mmHg
Highlights:

1. Elevated IOP seen in the uveitic glaucoma/OHT eyes (3-6 attacks), was due to reduced tonographic outflow facility

2. The aqueous humor flow rate was not detectibly different nor did the calculated uveoscleral outflow demonstrated any discernible difference.

3. Clinicians should consider treatment washout for those with medically treated uveitic glaucoma in the future

4. One should be mindful of the fact of the limitations of the current study. We calculated uveoscleral outflow by the Goldmann formula using assumed episcleral venous pressure. Additionally, we utilised indirect techniques to measure the aqueous flow rate and tonographic outflow facility. Consequently, these parameters may have been compromised as a result of inherent flaws of indirect techniques.