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In vivo biometric evaluation of Schlemm's canal with spectral-domain optical coherence tomography in pseuduexfoliation glaucoma

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ABSTRACT.

Purpose: **To perform *in vivo* imaging of the Schlemm's canal (SC) with anterior segment spectral-domain optical coherence tomography [AS-spectral-domain (SD)-OCT] and also to measure its biometric parameters including the SC length and SC area in patients with pseudoexfoliation (PEX) glaucoma.**

Methods: **Forty-one consecutive patients with PEX glaucoma and 41 age- and sex-matched normal subjects were enrolled. All subjects underwent imaging with SD-OCT. The SC length and SC area were examined in the temporal sections and measured with customized software.**

Results: **The percentages of the temporal sections in which SC was observable were similar between the two groups. Mean SC length was found significantly shorter, and mean SC area was found significantly smaller in patients with PEX glaucoma than in controls ($p = 0.044$ and $p = 0.036$, respectively). Mean intraocular pressure (IOP) values were also similar between two groups. No significant correlations were found between SC measurements and IOP.**

Conclusions: **Anterior segment spectral-domain optical coherence tomography offers non-invasive, *in vivo* measurement of the SC, and it could be used for investigating the SC changes in patients with PEX glaucoma.**

Key words: glaucoma – OCT – pseudoexfoliation – Schlemm's canal

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Introduction

Pseudoexfoliation (PEX) syndrome is biomicroscopically diagnosed by abnormal fibrillar deposits on ocular structures that line the aqueous-bathed surfaces of the anterior segment (Ritch & Schlötzer-Schrehardt 2001). Pseudoexfoliation predisposes to a number of ocular comorbidities, the most severe being glaucoma (Naumann et al. 1998). A previous histopathologic study of the Schlemm's canal (SC) width with light and electron microscopy in the eyes with PEX glaucoma revealed that the SC tends to become smaller in advanced cases (Johnson & Matsumoto 2000).

Anterior segment optical coherence tomography (AS-OCT) allows rapid *in vivo* non-contact qualitative and quantitative assessment of the human anterior chamber, and recent improvements in scan resolution have enabled the identification of aqueous outflow structures, including the SC (Asrani et al. 2008; Kagemann et al. 2010; Cheung et al. 2011; Usui et al. 2011).

Until now, *in vivo* evaluation of the SC in patients with PEX glaucoma has not been performed with any method. Therefore, we aimed to perform *in vivo* imaging of the SC with AS-spectral-domain (SD)-OCT and also to measure its biometric parameters including the SC length and SC area in patients with PEX glaucoma.

Patients and Methods

Participants

Forty-one consecutive patients with PEX glaucoma who visited our Department of Ophthalmology between June 2014 and September 2015 were enrolled into the study. Forty-one age- and sex-matched normal subjects were also studied, and one eye was randomly selected as the normal control.

Written informed consent was obtained from all participants. The study was approved by the local ethics committee and was performed in accordance with the tenets of the Declaration of Helsinki.

Inclusion criteria for PEX syndrome included those with evident classical dandruff or flaky exfoliation deposits on the pupil, lens or other ocular structures and radial pigment over the lens surface with or without raised

intra-ocular pressure (IOP) (Hong et al. 2014). Glaucoma was defined as those with glaucomatous optic neuropathy evidenced by cupping, rim thinning, notch or retinal nerve fibre layer defects with corresponding visual field defects (Hong et al. 2014). Subjects were classified as having PEX glaucoma if they had glaucoma and PEX in either eye. Participants in the control group were chosen from subjects with no complaints and normal ophthalmological examination.

Patients with any history of topical or systemic medication usage that could affect the drainage angle or pupillary reflex, previous intra-ocular surgery or laser treatment, ocular trauma, uveitis, corneal scars and any other pathology that could have led to secondary glaucoma were excluded from the study.

Examination

Comprehensive ophthalmic examinations were conducted including slit-lamp examination, applanation tonometry (Goldmann; Haag-Streit, Köniz, Switzerland), stereoscopic evaluation of the optic disc using a 90-dioptre lens (Volk optical, Inc., Mentor, OH, USA), measurements of axial length (AL) and anterior chamber depth (ACD) using the IOL Master (Carl Zeiss Meditec, Dublin, CA, USA), measurement of central corneal thickness (CCT) using ultrasound pachymetry AccuPach V (Accutome ultrasound, Inc., Malvern, PA, USA) and gonioscopy.

The morning IOP was measured between 07:00 and 09:30. It was analysed specifically because it is typically the time-point of the highest daily pressure (Stewart et al. 2013).

Five measurements of AL and ACD and three measurements of IOP and CCT were performed to calculate the mean values.

Medical/antiglaucomatous drug history and visual field test were used to assess the stages of clinical severity of glaucomatous damage, and eyes were grouped according to these stages (Johnson & Matsumoto 2000). Stage 1 eyes had mild disease, with a history of elevated IOP and either the use of one medication for treatment or no treatment; stage 2 eyes had a history of treatment with at least two medications, but did not have obvious disc damage or visual field loss; stage 3 eyes had mild-to-moderate visual field loss, confined to either upper or lower hemifields; and stage 4 eyes had advanced field loss in both the upper and lower hemifields.

Schlemm's canal measurements using spectral-domain optical coherence tomography

Before pupil dilation, all subjects underwent imaging with SD-OCT (RTvue OCT; Optivue Inc, Toledo, OH, USA) performed in dark room conditions by an experienced investigator (SI). The temporal limbus was imaged after adjustment of the fixation to the nasal area. During the examination, the subjects were encouraged to open their eyes as wide as possible, and if necessary, the examiner gently helped them to keep their eyes open using his fingers, taking care to avoid putting pressure on the eye. An eye speculum was not used. If the eye nictitation was noted during the examination, the examination was repeated up to three times (Usui et al. 2011).

Scans were obtained using the standard AS single scan protocol. In this mode, each scan included 1024 axial A scans with a total duration of 0.04 s on a 3-mm line at 3 o'clock position for optical density (OD) and 9 o'clock position for OS (Fig. 1). For the final B scan image (scan pattern: $x = 3$ mm and $z = 2$ mm), 16 B scan images (obtained from 1024 A scans) were automatically obtained and averaged to reduce speckle noise (from RTVue User's Manual provided by Optovue Inc, Fremont, CA, USA). Cross-sectional AS-OCT scans from the temporal section were acquired with the SD-OCT device. Two experienced investigators (SI and NYE) who were blind to the groups of subjects evaluated the images using customized software. On the averaged B scan images, SC length was defined as the avarage of the three measurements of the axial length of the thin, black and lucent space. The SC area was depicted as the area surrounded by the outline of SC (Hong et al. 2013). Schlemm's canal length and SC area were measured manually using only images in which SC was completely observable.

Statistical analysis

Statistical analyses were performed using the SPSS software version 16 (SPSS Inc., Chicago, IL, USA). The variables were investigated using visual (histograms, probability plots) and analytical methods (Kolmogorov–Smirnov/Shapiro-Wilk test) to determine whether or not they are normally distributed. Descriptive analyses were presented using means and standard deviations for normally distributed variables, and medians and interquartile range for the non-normally distributed variables.

Comparisons between groups were performed using the Student t-test and Mann–Whitney U-test. The chi-squared test or Fisher's exact test, where appropriate, was used to compare the percentages between groups. While investigating the associations between non-normally distributed and/or ordinal variables, the correlation coefficients and their significance were calculated using the Spearman test. A p value of less than 0.05 was considered statistically significant.

Accuracy for each SC measurement was tested using intraclass correlation coefficient (ICC) with the strength of agreement scale described by Brennan & Silman (1992). Accuracy was considered very good for ICC above 0.80

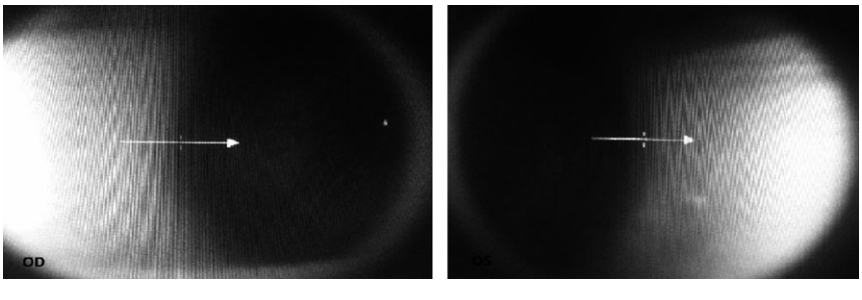

Fig. 1. Real-time optical coherence tomography image of the Schlemm's canal (SC) obtained with the line-scanning mode in the temporal quadrant after adjustment of the fixation to the nasal area.

and good for ICC between 0.61 and 0.80.

Interobserver reliability was evaluated by repetition of the SC measurements by an ophthalmologist (NYE) experienced in OCT blinded to previous measurements in a subsample of 20% of the main sample. These measurements and the originals were compared using ICC.

Intra-observer reliability was evaluated by repetition of the SC measurements by the main author blinded to previous measurements in 20% of subjects from the main sample. These pairs of measurements were compared using ICC.

Results

A total of 82 eyes from 82 subjects were enrolled into the study, including 41 patients with PEX glaucoma and 41 age- and sex-matched normal controls. The characteristics of the study subjects are shown in Table 1. There were no significant differences in IOP, AL and CCT between the patients and control group. However, a significantly low ACD was found in patients with PEX glaucoma when compared to control group (p = 0.002).

In the temporal sections, mean SC length was found significantly shorter and mean SC area was found significantly smaller in patients with PEX glaucoma than in controls (p = 0.044 and p = 0.036, respectively) (Table 2).

An oblong-elliptic/ovate-elliptic black space was visualized located close to the trabecular meshwork (TM) in most of the images generated using line scan mode (Fig. 2). The percentages of sections in which SC was observable and SC area was measurable in both groups are presented in Table 2. Patients with PEX glaucoma were grouped according to the glaucoma stages. Number of eyes for stage 1, 2, 3 and 4 were 12, 6, 18 and 5, respectively. The percentages of sections in which SC was observable in control group, patients with stage 1 + 2 and stage 3 + 4 PEX glaucoma were 87.8% (36/41), 76.5% (13/17) and 62.5% (15/24), respectively (p = NS). However, the percentages of sections in which SC area was measurable in control group, patients with stage 1 + 2 and stage 3 + 4 PEX glaucoma were 82.9% (34/41), 76.5% (13/17) and 54.2% (13/24), respectively (p = 0.039).

Table 1. Characteristics of the study subjects.

Parameters	PEXG patients (*n*: 41)	Controls (*n*: 41)	p
Age (years)	66.5 (8.7)	64.7 (7.6)	NS
Gender (M:F)	23:18	23:18	NS
IOP (mmHg)	16 (13–19.5)	15 (13–18)	NS
CCT (μm)	546.9 (35.5)	549.6 (31.4)	NS
AL (mm)	23.1 (0.7)	23.3 (0.7)	NS
ACD (mm)	2.8 (0.3)	3.0 (0.2)	0.002
Number of glaucoma drugs	2 (1–3)	–	–

Values were presented as mean (standard deviation) and median (25–75%). PEXG, pseudoexfoliation glaucoma; IOP, intra-ocular pressure; CCT, central corneal thickness; AL, axial lenght of the eye; ACD, anterior chamber depth.

Table 2. Comparison of Schlemm's canal parameters in patients with pseudoexfoliation glaucoma and normal controls.

Parameters	PEXG patients (*n*: 41)	Controls (*n*: 41)	p
SC lenght (μm)	301.3 (84.1)	349.3 (86.8)	0.044
SC area (μm^2)	4000 (3000–5000)	5000 (3000–7750)	0.036
Subjects with observable SC (*n*)	29 (70.7%)	36 (87.8%)	NS
Subjects with measurable SC area (*n*)	26 (63.4%)	34 (82.9%)	0.046

Values were presented as mean (standard deviation) and median (25–75%). PEXG, pseudoexfoliation glaucoma; SC, Schlemm's canal.

Both SC length and SC area did not correlate with the other parameters including IOP, glaucoma stages, AL, ACD and CCT.

Interobserver reliability was very good for SC length and SC area, with ICCs of 0.90 and 0.81, respectively. Intra-observer reliability was also very good for SC length and SC area, with ICCs of 0.98 and 0.84, respectively.

Discussion

To our knowledge, this is the first *in vivo* study evaluating biometric parameters of SC in patients with PEX glaucoma with AS-SD-OCT. Both mean SC length and SC area were found to be significantly decreased in patients with PEX glaucoma when compared to controls.

Irshad et al. (2010) performed *in vivo* investigation of SC diameter in glaucoma patients with ultrasound biomicroscopy (UBM). Spectral/Fourier domain AS-OCT provides cross-sectional views of the AS structures with a resolution better than that of UBM. Images and measurements of very fine structures of the AS can be achieved rapidly and non-invasively (Zheng et al. 2011). Fuest et al. (2015) evaluated early anatomical changes following canaloplasty with AS-SD-OCT and UBM. They concluded that AS-OCT offers a high resolution for imaging superficial conjunctival areas and SC after canaloplasty, whereas UBM is capable of detecting deeper structures such as scleral lakes and intra-canal sutures.

In the literature, there are AS-OCT studies evaluating *in vivo* features of SC in primary open-angle and angle-closure glaucoma (Hong et al. 2013; Rao & Padhy 2014). In a study by Kagemann et al. (2010), SC areas imaged with SD-OCT were found significantly smaller in glaucoma patients than in control subjects, leading to the possibility that some of the findings may be unrelated to the PEX material. We did not include a group with primary open-angle glaucoma. This was a major limitation of our study.

Usui et al. (2011) identified SC and its surrounding tissues in enucleated human eyes by AS-Fourier domain-OCT and histologic examination. Schlemm's canal length was determined as 354 μm on the OCT image and 384 μm on histologic sections. Similarly, in our study, the mean SC length was 349 μm. The SC length measured *in vivo* may reflect the canal's functional size more accurately because of partial collapse of the canal secondary to physiological conditions (Irshad et al. 2010).

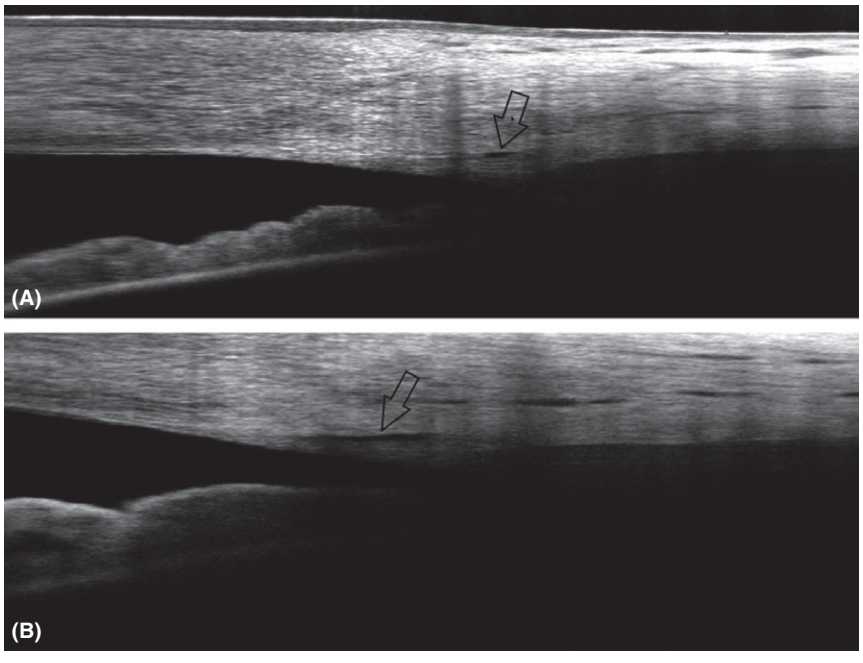

Fig. 2. Sectional images of the Schlemm's canal (SC) (arrows) by anterior segment optical coherense tomography in (A) a patient with pseudoexfoliation glaucoma and (B) in a healthy control.

As IOP increases, the TM expands into the lumen of the SC and causes a concomitant narrowing of the lumen (Johnstone & Grant 1973). In another study by Kagemann et al. (2014a,b), they reported that IOP elevation in healthy subjects reduces SC cross-sectional area. In our study, however, the mean IOP values were similar in PEX glaucoma group and in controls. Also, we did not find any correlation between IOP and SC measurements.

Shi et al. (2014) studied the morphological changes in SC in treated and newly diagnosed untreated glaucomatous eyes by swept source OCT. In their study, the area, circumference and long diameter of SC were found smaller in untreated patients than in treated patients. They stated that after the drug therapy, the IOP would be reduced, which could lead to expansion of the SC, causing the area and circumference to become larger. As our sample size was small and the number of newly diagnosed untreated cases was low, we could not determine the morphological changes in the SC with antiglaucomatous drug treatment. This was a limitation of our study.

In this study, the investigators were confident that the SC could be observed in up to 87% of scans in control group and 70% in PEX glaucoma group. Consistent with the results of our study, Usui et al. (2011) reported that the SC was observable in 90% of normal cases. On the other hand, Kagemann et al. (2010) identified the SC in 100% of normal subjects.

In the literature, it is reported that the gradual build-up of PEX material in the juxtacanalicular tissue correlates with the IOP level and with the presence and severity of glaucomatuos optic nerve damage and may be associated with progressive degenerative changes of SC including narrowing, fragmentation and obstruction in more advanced cases (Schlötzer-Schrehardt & Naumann 1995, 2006; Gottanka et al. 1997). In our study, the percentage of PEX glaucoma patients with measurable SC area was 63%, whereas it was 82% in control group ($p = 0.046$). This significant difference between the percentages of the measurable SC area in two groups and narrowing of SC in patients with PEX glaucoma could be explained, in part, by the accumulation of PEX material in the juxtacanalicular tissue. Johnson et al. (2000) studied the TM and SC with light and electron microscopy in patients with PEX syndrome or PEX glaucoma. They reported that eyes with PEX tended to have a smaller canal with increasing severity of disease. However, they studied only two eyes with advanced PEX glaucoma. In this study, the percentage of measurable SC area in eyes with advanced PEX glaucoma (stage 3 + 4) was significantly lower (54%) than that in eyes with early PEX glaucoma (stage 1 + 2) (76%) and that in control eyes (82%) ($p = 0.039$).

Hong et al. (2013) used SD-OCT to compare SC area in healthy eyes and those with primary open-angle glaucoma in cohorts of Chinese persons. They used a single line scan, interrogating the limbus until a clear view of SC was obtained. Buller and Johnson (1994) examined the variability of the TM in normal and glaucomatous eyes with light and electron microscopy. Significant segmental differences were not found within single eyes in both groups and they stated that differences among quadrants were qualitative rather than quantitative. Usui et al. (2011) studied SC length, TM length and TM area in enucleated human eyes by AS-Fourier domain-OCT. Differences in parameter measurements were not statistically significant between temporal and nasal sections and between right and left eyes. On the other hand, previous studies reported that the SC area can vary dramatically in different scan locations, even in a small distance (Kagemann et al. 2010, 2014a,b). It is also emphasized that to describe SC area, a high density of radial scans facilitating multiple measurements of SC area, as provided by a volumetric scan across the region of interest, is required (Kagemann et al. 2014a,b). Therefore, whether a single scan measurement could represent the real SC requires further investigation. This could be another limitation of our study.

In conclusion, AS-SD-OCT offers non-invasive, *in vivo* measurement of the SC and it could be used for investigating the SC changes in patients with PEX glaucoma.

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