In Vivo Qualification of Human Aqueous Veins by EDI-OCT and OCTA Images

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Research Article

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Abstract

PURPOSE.

To investigate the aqueous vein in vivo by using enhanced depth imaging optical coherence tomography (EDI-OCT) and optical coherence tomography angiography (OCTA).

METHODS.

In this cross-sectional comparative study, 30 healthy participants were enrolled. Images of the aqueous and conjunctival veins were captured by EDI-OCT and OCTA during the static state and after water loading. The area, height, width, depth and blood flow of the aqueous vein and conjunctival vein were measured by ImageJ software.

RESULTS.

In the static state, the area of the aqueous vein was $8166.7 \pm 3272.7 \mu m^2$, which was smaller than that of the conjunctival vein ($13690 \pm 7457 \mu m^2$). The mean vessel density of the aqueous vein was $35.3 \pm 12.6\%$, which was significantly less than that of the conjunctival vein ($51.5 \pm 10.6\%$). After water loading, the area of the aqueous vein decreased significantly from $8725.8 \pm 779.4 \mu m^2$ (baseline) to $7005.2 \pm 566.2 \mu m^2$ after 45 min but returned to $7863.0 \pm 703.2 \mu m^2$ after 60 min. The vessel density of the aqueous vein decreased significantly from $41.2 \pm 4.5\%$ (baseline) to $35.4 \pm 3.2\%$ after 30 min but returned to $45.6 \pm 3.6\%$ after 60 min.

CONCLUSIONS.

The structure and vessel density of the aqueous vein can be effectively evaluated by OCT and OCTA, which might provide a biological indicator to evaluate aqueous vein changes and aqueous outflow resistance under different interventions in glaucoma patients.

Introduction

The aqueous humor drains via the trabecular meshwork into Schlemm's canal, the canal then directs the aqueous humor to a network of aqueous veins in the conjunctiva and aqueous venous plexuses and, finally, to the aqueous veins$^{1-2}$. The study of biological characteristics of the aqueous humor vein plays an important role in observing the regularity of aqueous humor outflow, while the physiology of the aqueous vein remains poorly understood.
The aqueous veins are biomicroscopically visible pathways with a blood vessel-like appearance containing clear colorless blood, diluted blood or both\(^3\)-\(^5\). The transparency of the aqueous humor makes it difficult to observe the aqueous vein, and the aqueous vein is easily confused with conjunctival vessels. Aqueous veins were mentioned by Leber as early as 1903, and previous studies on aqueous veins have mainly relied on slit lamp microscopy and casting studies\(^6\)-\(^11\), which were too subjective and rudimentary to reflect the characteristics of aqueous veins in vivo. The detailed morphologic and functional status of the aqueous veins remains unknown. Optical coherence tomography (OCT) is a label-free imaging technique that measures depth-resolved tissue reflectance, achieving 3D imaging at micrometer-scale resolutions (typically 5-20 \(\mu\)m). OCT clearly shows the structure of the limbus, including the small Schlemm's canal\(^12\)-\(^13\). OCT angiography (OCTA) has been applied to image blood vessels in various tissues, such as the eye, brain and skin, and OCTA clearly images the fundus and conjunctival vessels\(^14\)-\(^15\). However, there is a lack of OCT and OCTA studies on aqueous veins.

In this study, OCT and OCTA imaging of the aqueous vein was performed on normal subjects, and the differences in the structure and blood flow between the aqueous vein and the accompanying conjunctival vein were compared. At the same time, a water-drinking test was performed to observe the dynamic changes in the aqueous vein during the process of IOP changes.

**Methods**

**Ethics Statement**

The study protocols were approved by the Ethics Committee of Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, China. Written informed consent was obtained prior to enrollment from all the participants, and the study was performed in accordance with the tenets set forth in the Declaration of Helsinki.

**Study Subjects**

Thirty healthy volunteers from Tongji Hospital in Wuhan (Hubei Province, China) were recruited between October 2020 and December 2020. A single eye was enrolled randomly for all subjects.

The inclusion criteria were as follows: 1) age > 18 years; 2) IOP : 10-21 mmHg, best-corrected visual acuity (BCVA) \(\geq 20/20\), refractive error (RE) > -6.0 D and RE < +3.0 D, and normal ophthalmoscopic; 3) no history of other eye diseases, such as macular degeneration, retinal detachment; 4) no use of medication that affects the circulatory system within the month prior to enrollment; 5) no history of hypertension, diabetes.

**Water-Drinking Test and Imagine Processing**
Patients fasted for at least 4 hours and six measurement timepoints at baseline and 0, 15, 30, 45 and 60 min after drinking 1 liter of water within 5 min were scheduled. The IOP was measured using a noncontact tonometer (NCT, NT-530P, Nidek Co., LTD). Three measurements were obtained, and the average value was recorded.

Conjunctival vessels were used as landmarks to scan the same area, judging from the coordinates from corneal limbus. The structure of the aqueous vein and accompanying conjunctival vein were captured at six timepoints using a Spectralis enhanced depth imaging optical coherence tomography (EDI-OCT) device (Heidelberg Engineering GmbH, Dossenheim, Germany) after resting for 5 min. The following parameters were measured: area of the aqueous vein and conjunctival vein; height of the aqueous and conjunctival veins (coronal diameter, μm); width of the aqueous and conjunctival veins (meridional diameter, μm); and depth of the aqueous and conjunctival veins (vertical diameter to the ocular surface, μm) (Figures 1 and 2).

**OCTA Imaging**

OCTA images of the aqueous and conjunctival veins were acquired using a Spectralis OCT device (SPECTRALIS®, Heidelberg Engineering, Heidelberg, Germany). Images were acquired with an A-scan rate of 70,000 per second, and a 10°× 5° scan angle protocol was used. A total of 128 B-scans resulted in images with an axial resolution of approximately 4 μm within a B-scan resolution of approximately 9 μm (3.87 μm/pixel). The scanning frame dimensions were 2.4 × 1.2 mm. All OCT and OCTA tests were performed under standardized darkroom photopic conditions (ca. 3.5 lux).

**Data processing**

The area, height, width and depth of the aqueous and conjunctival veins (μm²) as well as the vessel density of the aqueous vein (AVD, %) and conjunctival vein (CVD, %) were measured at six time points during the water-drinking test using ImageJ software (version 1.53a; National Institutes of Health, Bethesda, MD, USA). The grayscale of the aqueous and conjunctival veins (pixels) was calculated by the mean Integrated Density Analysis of ImageJ software (Figures 3). Analysis of AVD and CVD was performed using the Vessel Analysis plugin (Figures 4 and 5). Vascular density was calculated by the Vessel Analysis plugin of ImageJ software (Equation 1) as follows:

\[
Vascular\ density = \frac{\text{Vessel area}}{\text{Total area}} \times 100\%
\]  

Equation 1

The measurements were performed by two observers (Z.Q.C. and W.C.), and cases of discrepancy >15% were resolved by consulting the senior author (J.M.W.). Data were recorded and stored for later statistical analysis.

**Statistical Analysis**
All statistical analyses were performed using SPSS software (Version 25.0, SPSS Inc., Chicago, IL, USA). Data are presented as the mean values ± standard deviations (SDs). Student’s t test was used to compare the differences of aqueous and conjunctival veins in area, height, width and depth. One-way repeated-measures analysis of variance was used to compare variations in the area and vessel density of the aqueous and conjunctival veins at different time periods (baseline and every 15 min for 1 hour). All tests were two-tailed. Statistical significance was defined as a P-value < 0.05.

**Results**

Thirty eyes from 30 healthy participants (13 males and 17 females) aged 22 to 38 years (27.8 ± 4.1 years, mean ± SD) were included in the study. The mean axial length was 25 ± 0.8 mm, and the mean IOP was 15.3 ± 3.6 mmHg before the procedure as measured by a noncontact tonometer (Table 1). Twenty-six participants completed the water-drinking test.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Values</th>
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</thead>
<tbody>
<tr>
<td>Number of subjects</td>
<td>30</td>
</tr>
<tr>
<td>Number of eyes</td>
<td>30</td>
</tr>
<tr>
<td>Age (year)</td>
<td>27.8 ± 4.1</td>
</tr>
<tr>
<td>Sex (NO.)</td>
<td>17</td>
</tr>
<tr>
<td>Female</td>
<td>13</td>
</tr>
<tr>
<td>Male</td>
<td></td>
</tr>
<tr>
<td>IOP (mmHg)</td>
<td>15.3 ± 3.6</td>
</tr>
<tr>
<td>Refractive error (diopter)</td>
<td>-3.8 ± 2.3</td>
</tr>
<tr>
<td>Axial length (mm)</td>
<td>25 ± 0.8</td>
</tr>
</tbody>
</table>

The mean area of aqueous vein was 8166.7 ± 3272.7 µm², which was smaller than that of the conjunctival vein (13690 ± 7457 µm²). The mean height of the aqueous vein was 81.3 ± 19.2 µm, which was lower than that of the conjunctival vein (103.4 ± 29.6 µm). The mean width of the aqueous vein as 106.7 ± 25.0 µm, which was narrower than that of the conjunctival vein (158 ± 45.8 µm). The mean depth of the aqueous vein was 212.7 ± 46.2 µm, which was the same as that of the conjunctival vein (209.8 ± 46.4 µm). The mean vessel density of the aqueous vein was 35.3 ± 12.6%, which was significantly decreased compared to that of the conjunctival vein (51.5 ± 10.6%). The grayscale of the aqueous vein (112.9 ± 24.9 pixels) was higher than that of the conjunctival vein (79.6 ± 25.5 pixels) (Fig. 6).

The IOP was 14.9 ± 0.7 mmHg at baseline, increased to 17.6 ± 0.8 mmHg at 15 min after the water-drinking test and then returned to 15.1 ± 0.7 mmHg at 60 min after the water-drinking test. The area of the
aqueous vein decreased significantly from $8725.8 \pm 779.4 \mu m^2$ (baseline) to $7005.2 \pm 566.2 \mu m^2$ after 45 min but returned to $7863.0 \pm 703.2 \mu m^2$ after 60 min (Fig. 7). The vessel density of the aqueous vein decreased significantly from $41.2 \pm 4.5\%$ (baseline) to $35.4 \pm 3.2\%$ after 30 min but returned to $45.6 \pm 3.6\%$ after 60 min (Fig. 7).

There were no significant differences in the height, width or depth of the aqueous vein during water loading.

**Discussion**

Currently, data on the aqueous vein in vivo are limited; therefore, we investigated the presence of aqueous veins using OCT and OCTA images in this study. To our knowledge, this is the first in vivo study to report OCT and OCTA manifestations of aqueous veins.

There were many studies on aqueous veins in the 1950s and 1960s\textsuperscript{6-9}. In vitro casting studies have been generally used to observe the course and morphological characteristics of aqueous veins. In vivo studies on the aqueous vein have been mainly conducted by direct observation with a slit lamp microscope, and the size, contour and location of the aqueous vein could be evaluated in the photograph of the slit lamp. Recently, two studies described a technique to noninvasively visualize aqueous veins using hemoglobin video imaging (HVI) technology, which uses the hemoglobin absorption spectrum to enhance the contrast between red blood cells and their surroundings\textsuperscript{10-11}. However, the image analysis using HVI technology is still based on slit lamp images, and it is not possible to accurately evaluate aqueous veins. In the present study, OCT was used to objectively observe the aqueous vein. Multiple parameters were accurately measured and compared with the morphological characteristics of the conjunctival vein, which helped to accurately identify aqueous veins according to these parameters. In addition, these parameters provided biological indicators for evaluation of aqueous vein changes under different interventions.

OCT angiography (OCTA) is an emerging technology for imaging ocular vasculature, and it works on the concept of low coherence interferometry and analysis of signal decorrelation between consecutive scans. In addition to the fundus vessels, OCTA is also used to observe the conjunctiva, superficial sclera and iris vessels. OCTA visualizes blood flow in vessels through motion contrast imaging of erythrocyte movement across sequential B-scans\textsuperscript{17-18}. Because the aqueous vein contains different amounts of blood and aqueous humor, we used OCTA to observe the aqueous vein in the present study, which allowed the content of aqueous humor in the aqueous vein to be inferred from the change in OCTA signals. In the present study, we found that the vessel density of the aqueous vein was significantly lower than that of the conjunctival vein, suggesting that the blood flow of the aqueous vein is less than that of the conjunctival vein. These findings agreed with the anatomical and physiological characteristics of the aqueous vein and conjunctival vein, which confirmed the reliability of OCTA to observe the blood flow of the aqueous vein.
After the water-drinking test, the IOP increased, reaching a peak 15 min after water loading, and it then gradually decreased and returned to the normal level 60 min after water loading, which was consistent with the results reported in previous literature\textsuperscript{19–21}. Our results showed that the area of the aqueous vein decreased after the water-drinking test, which occurred at a minimum of 15 min after water-loading followed by a gradual increase to a normal level at 60 min after water-loading. We speculated that the changes of the aqueous vein area after the water-drinking test were intrinsic and related to the changes of IOP. Previous studies have shown that the aqueous humor flow is at a minimum approximately 10 min after the water-drinking test\textsuperscript{21}, which may be directly related to the highest IOP and the lowest aqueous vein area 15 min after water-loading. However, whether the decrease in aqueous vein area after the water-drinking test is one of the reasons for the decrease in aqueous humor outflow and the increase in IOP remains to be further explored. In addition, our results showed that the aqueous vein density decreased after the water-drinking test, which may be related to the decrease in the aqueous vein area, leading to a decrease in the total amount of aqueous humor and blood in the aqueous vein lumen.

OCT is an imaging modality that provides cross-sectional images based on the measurement of the magnitude and echo time delay of back-scattered light\textsuperscript{22}. Quantification of different reflective bands of OCT images has been widely used in the retina diseases and cataract\textsuperscript{23–24}, but no OCT reflectivity of the aqueous vein has been reported. In our study, the aqueous vein was observed as a rounded, gray black, lucent space on the OCT images. The grayscale of the aqueous vein was larger than that of the conjunctival vein, and the OCT reflectivity of the aqueous vein was much lower, which may be due to more water in the aqueous vein than in the conjunctival vein. This new information is of value for the interpretation of aqueous vein OCT images and may assist the confirmation of aqueous veins in future studies.

The aqueous veins are not distributed symmetrically around the limbus. Two to three aqueous veins are typically visible in an eye, but there may be a maximum of four to six, the distribution is highly asymmetric with the majority of visible aqueous veins at or below the horizontal midline\textsuperscript{25–26}. In the present study, the most obvious aqueous vein accompanied by the conjunctival vein was selected as the object of observation. The aqueous vein was observed in the inferior quadrant in 23 of the 30 enrolled subjects, and the distribution was consistent with previous reports. Our study confirmed that the aqueous vein in the inferior quadrant was more obvious and could be used as the main site for future research on the aqueous vein.

The present study had several limitations. First, the aqueous vein was investigated only in healthy subjects who were approximately 30 years old, and the morphology and blood flow of the aqueous vein may differ between elderly and young subjects. Second, the aqueous vein observed in this study was mostly in the inferior quadrant, and there might be a difference between the different quadrants of the aqueous vein. Third, the scan line of the conjunctival vein in this study was not necessarily perpendicular to the pipe of the conjunctival vein, resulting in some deviation in the measurement of the conjunctival vein. However, the main object of this research was the aqueous vein, and the angle between the scan line...
and conjunctival vein should be adjusted in future research on the conjunctival vein. Finally, because the study sample size was calculated for aqueous vein changes as the primary outcome, it may have been underpowered to detect an association between changes in IOP and the aqueous vein after the water-drinking test.

In summary, our study objectively demonstrated a significant observation of the aqueous vein in healthy subjects, showing that OCT coupled with OCTA analysis can be used as a practical tool for effectively evaluating aqueous vein structure and function. The present study provided a potential new method to evaluate the pathophysiology of glaucoma patients in the future.

Declarations

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Authors’ contributions

Zhiqi Chen, Wei Chen, Chaohua Deng and Jingmin Guo performed the research and data analysis, Zhiqi Chen and Wei Chen wrote the main manuscript text, HongZhang and Junming Wang designed the study and revised the manuscript. All authors reviewed the manuscript.

Ethics approval and consent to participate

This observational study was approved by the ethics committee of Tongji Hospital (Registration Number: TJ-IRB20201024) and adhered to the tenets of the Declaration of Helsinki. All subjects provided written informed consent prior to study participation.

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Consent for publication

Not applicable.

Competing Interests: The authors declare no competing interests.

References


Figures

![A](image1.png)

![B](image2.png)
**Figure 1**

A, 1B: Examples of the structure of AV (red circle) and accompanying CV (green circle) (cases 1 and 2). AV, aqueous vein; CV, conjunctival vein.

**Figure 2**

Measurement of area, depth, width and height of AV and CV. AV, aqueous vein; CV, conjunctival vein.
Figure 3

The structural variations of AV at different timepoints during the water-drinking test. AV, aqueous vein.

Figure 4
OCTA image of AV and CV. AV, aqueous vein; CV, conjunctival vein.

**Figure 5**

Vessel density variations in OCTA images of the AV during the water-drinking test. AV, aqueous vein.

**Figure 6**

Comparison of architectures between the aqueous vein and conjunctival vein.
Figure 7

Variations in the parameters of the aqueous vein during the water-drinking test.