· Clinical Research ·

In vivo quantification of human aqueous veins by enhanced depth imaging optical coherence tomography and optical coherence tomography angiography images

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Abstract

● **AIM:** 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 before and after water loading. The area, height, width, location depth and blood flow of the aqueous vein and conjunctival vein were measured by Image J software.

● **RESULTS:** In the static state, the area of the aqueous vein was 8166.7±3272.7 μm^2, which was smaller than that of the conjunctival vein (13 690±7457 μm^2, P<0.001). The mean blood flow density of the aqueous vein was 35.3%±12.6%, which was significantly less than that of the conjunctival vein (51.5%±10.6%, P<0.001). After water loading, the area of the aqueous vein decreased significantly from 8725.8±779.4 μm^2 (baseline) to 7005.2±566.2 μm^2 at 45min but rose to 7863.0±703.2 μm^2 at 60min (P=0.032). The blood flow density of the aqueous vein decreased significantly from 41.2%±4.5% (baseline) to 35.4%±3.2% at 30min but returned to 45.6%±3.6% at 60min (P=0.021).

● **CONCLUSION:** The structure and blood flow density of the aqueous vein can be effectively evaluated by OCT and OCTA. These may become biological indicators to evaluate aqueous vein changes and aqueous outflow resistance under different interventions in glaucoma patients.

● **KEYWORDS:** aqueous vein; optical coherence tomography; optical coherence tomography angiography; water drinking test

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INTRODUCTION

The aqueous humor drains *via* the trabecular meshwork into Schlemm's canal, and the canal then directs the aqueous humor to a network of collector channels and, finally, to the aqueous veins[1-2]. The study of the biological characteristics of the aqueous vein plays an important role in observing the regularity of aqueous humor outflow, but 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 aqueous humor, diluted blood, or both[3-5]. The transparency of the aqueous humor makes it difficult to observe the aqueous vein, so the aqueous vein is easily confused with conjunctival vessels. Aqueous veins were mentioned by Leber as early as 1903, and studies on aqueous veins have mainly relied on slit lamp microscopy and casting studies for visualization[6-11], but these methods are too subjective and rudimentary to reflect the characteristics of aqueous veins *in vivo*. The aqueous veins have not been characterized in detail morphologically or functionally. 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 μm). OCT clearly shows the structure of the limbus, including the small Schlemm's canal[12-13]. Optical coherence tomography angiography (OCTA) has been applied to image blood vessels in various tissues, such as the eye, brain, and skin, as it clearly images the fundus and conjunctival vessels[14-15]. 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 human subjects, and the structure and blood flow were compared between the aqueous vein and the accompanying conjunctival vein. In addition, a water-drinking test was performed to observe the dynamic changes in the aqueous vein during the process of intraocular pressure (IOP) change.

SUBJECTS AND METHODS

Ethical Approval The study protocols were approved by the Ethics Committee of Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, China (TJ-IRB20201024). Written informed consent was obtained prior to enrollment from all 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 of each subject was enrolled randomly.

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

Water-Drinking Test and Image Processing Patients fasted for at least 4h. Six measurement timepoints, at baseline and 0, 15, 30, 45, and 60min after drinking 1 liter of water over ≤5min, were scheduled. The IOP was measured using a noncontact tonometer (NCT, NT-530P, Nidek Co., LTD, Japan). Three measurements were taken, and their average value was recorded.

The aqueous veins are not distributed symmetrically around the limbus. The inferior nasal quadrants are most commonly found in the aqueous vein. In our study, the inferior quadrants were the key area we chose to find the aqueous vein. The scan of the aqueous vein was done 2 mm from the limbus, and the scan frame was perpendicular to the direction of the aqueous vein. The same site was scanned at all time points in the same subject. The most obvious aqueous vein of one eye of each subject was enrolled. The characteristics of the aqueous vein were mainly as follows: 1) Aqueous veins generally contain clear aqueous humor drained from the collecting canal. 2) Laminal flow of aqueous humor and small amounts of blood that drained from the episcleral vein were observed in aqueous veins. Conjunctival vessels were used as landmarks to scan the same area, judging from the coordinates of the corneal limbus. The structures of the aqueous vein and accompanying conjunctival vein were captured at the six time points using a Spectralis enhanced depth imaging optical coherence tomography (EDI-OCT) device (Heidelberg Engineering GmbH, Dossenheim, Germany) after resting for 5min. The following parameters were measured: area of the aqueous vein and conjunctival vein (μm^2); height of the aqueous and conjunctival veins (coronal diameter, μm); width of the aqueous and conjunctival veins (meridional diameter, μm); and location depth of the aqueous and conjunctival veins (vertical diameter to the ocular surface, μm; Figures 1 and 2).

Optical Coherence Tomography Angiography 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^2. The depth of OCTA images was 1.9 mm (3.87 μm/pixel). The SPECTRALIS OCTA images have a binary, high-contrast appearance. Increasing the number of repeated scans at each location increases the contrast between perfused vessels and static tissue. Seven repeated scans were done in our study to yield high-quality OCTA images. The contrast of OCTA can be varied between 4-7 repeated scans. The SPECTRALIS OCTA algorithm is a full-spectrum probabilistic approach. The algorithm computes the probability that a given pixel follows the OCT signal distribution of the perfused flow rather than the distribution of static tissue. Given these distributions and a short time series of samples (4 to 7 repeated scans), it is possible to determine the probability of whether the signal at that sample location corresponds to one of the two distributions. All OCT and OCTA tests were performed under standardized darkroom photopic conditions (3.5 lx).

Data Processing The area, height, width and location depth of the aqueous and conjunctival veins (μm^2) as well as the blood flow density of the aqueous vein (AVD, %) and conjunctival vein (CVD, %) were measured at the six time points during the water-drinking test using Image J software (version 1.53a; National Institutes of Health, Bethesda, MD, USA). The grayscale of the aqueous and conjunctival veins (pixels) was calculated by mean integrated density analysis in Image J software (Figure 3). Analysis of AVD and CVD was performed using the Vessel Analysis plugin (Figures 4 and 5). Blood flow density was calculated by the vessel analysis plugin (Equation 1) as follows[16]:

$$\text{Blood flow density} = \frac{\text{flow signal area}}{\text{Total area}} \times 100\% \qquad (1)$$

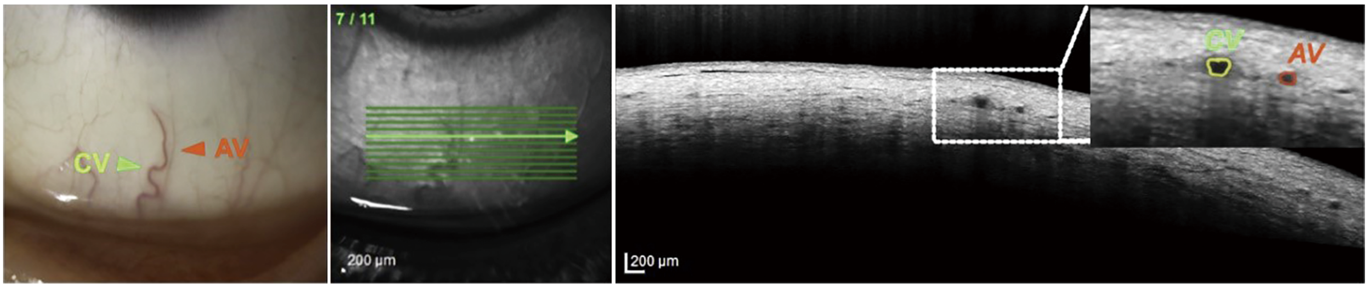

Figure 1 Examples of the structure of AV (red circle) and accompanying CV (green circle) AV: Aqueous vein; CV: Conjunctival vein.

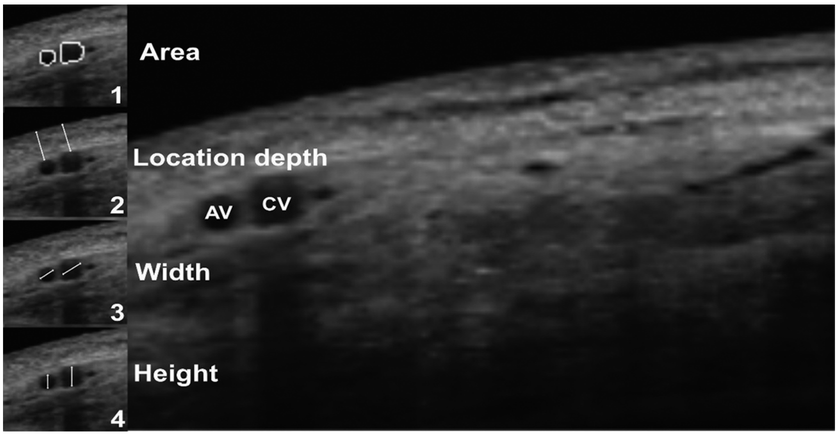

Figure 2 Measurement of the area, location depth, width and height of the aqueous vein (AV) and conjunctival vein (CV).

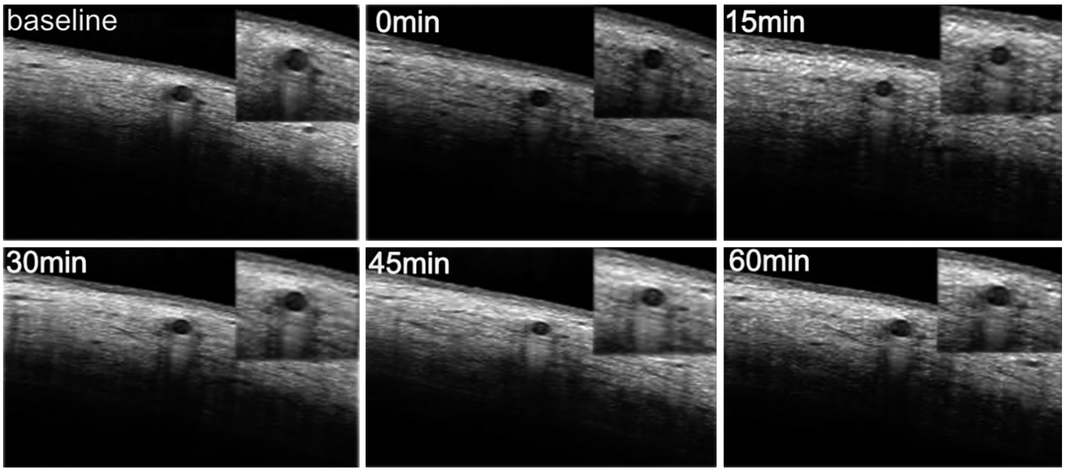

Figure 3 The structural variations in AV at different timepoints during the water-drinking test AV: Aqueous vein.

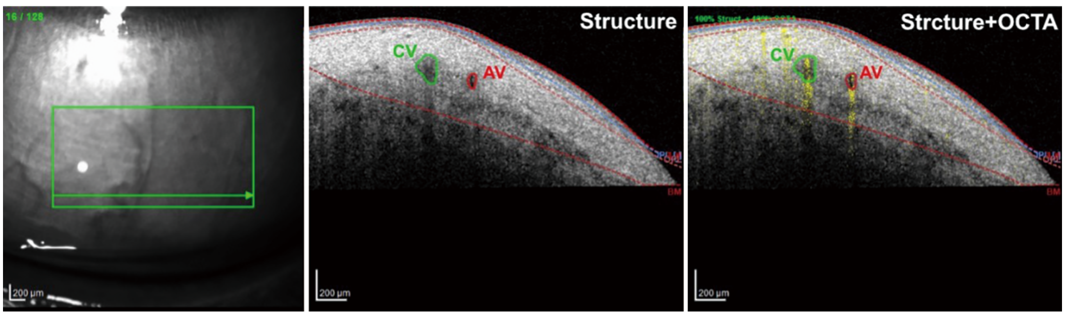

Figure 4 Optical coherence tomography angiography image of the aqueous vein (AV) and conjunctival vein (CV).

The measurements were performed by two observers (Chen ZQ and Chen W). Cases of discrepancy >15% were resolved by consulting the senior author (Wang JM). 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 mean±standard deviation (SD). Student's *t*-test was used to compare the differences in the area,

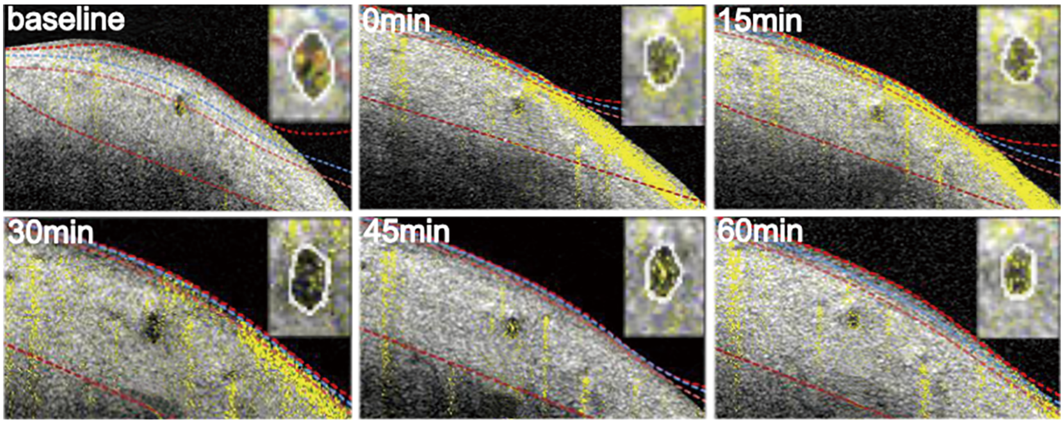

Figure 5 Blood flow density variations in optical coherence tomography angiography images of the aqueous vein (AV) during the water-drinking test.

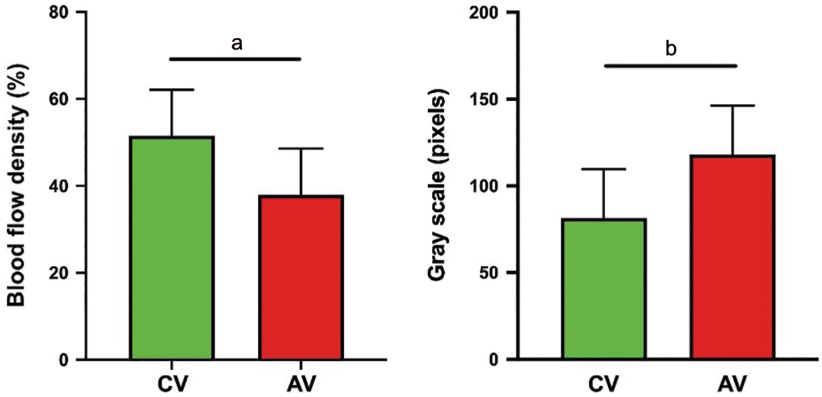

Figure 6 Comparison of architecture between the aqueous vein and conjunctival vein CV: Conjunctival vein; AV: Aqueous vein. [a]*P*<0.01, [b]*P*<0.001.

height, width, and depth of the aqueous and conjunctival veins. One-way repeated-measures analysis of variance was used to compare variations in the area and blood flow density of the aqueous and conjunctival veins at the different time points (baseline and every 15min for 1h). 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 38y (27.8±4.1y) were included in the study. The mean axial length was 25±0.8 mm, and the mean IOP was 15.3±3.6 mm Hg before the procedure, as measured by a noncontact tonometer (Table 1). Twenty-six participants completed the water-drinking test.

Table 1 Baseline characteristics

Parameters	Values
No. of subjects	30
No. of eyes	30 (13 right eyes and 17 left eyes)
Age (y)	27.8±4.1
Sex (*n*)	
Female	17
Male	13
IOP (mm Hg)	15.3±3.6
Refractive error (D)	-3.8±2.3
Axial length (mm)	25±0.8

D: Diopter; IOP: Intraocular pressure.

The mean area of the aqueous vein was 8166.7±3272.7 μm^2, which was smaller than that of the conjunctival vein (13 690±7457 μm^2). 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 was 106.7±25.0 μm, which was narrower than that of the conjunctival vein (158±45.8 μm). The mean location depth of the aqueous vein was 212.7±46.2 μm, which was not different from that of the conjunctival vein (209.8±46.4 μm). The grayscale value of the aqueous vein (112.9±24.9 pixels) was higher than that of the conjunctival vein (79.6±25.5 pixels). The mean blood flow density of the aqueous vein was 35.3%±12.6%, which was significantly lower than that of the conjunctival vein (51.5%±10.6%; Figure 6).

The IOP was 14.9±0.7 mm Hg at baseline, increased to 17.6±0.8 mm Hg at 15min after the water-drinking test, and then returned to 15.1±0.7 mm Hg at 60min after the water-drinking test. The area of the aqueous vein decreased significantly from 8725.8±779.4 μm^2 (baseline) to 7005.2±566.2 μm^2 after 45min but rose to 7863.0±703.2 μm^2 after 60min (Figure 7). The blood flow density of the aqueous vein decreased significantly from 41.2%±4.5% (baseline) to

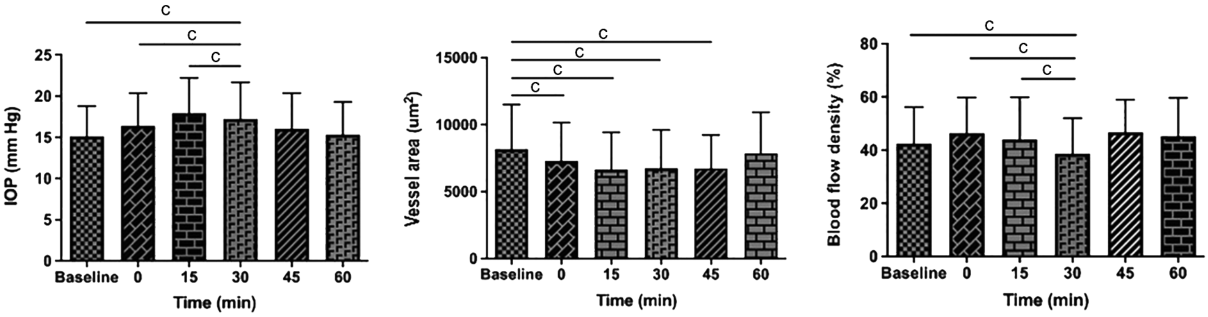

Figure 7 Variations in the parameters of the aqueous vein during the water-drinking test IOP: Intraocular pressure. $^cP<0.05$.

35.4%±3.2% after 30min but returned to 45.6%±3.6% after 60min (Figure 7).

There were no significant changes in the height, width, or location depth of the aqueous vein during water loading (all $P>0.05$). There was no significant correlation between the variation in vessel area and the variation in IOP ($P=0.11$).

DISCUSSION

The aqueous vein has been little studied *in vivo*, so 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 the OCT and OCTA manifestations of aqueous veins.

There were many studies on aqueous veins in the 1950s and 1960s[6-9]. *In vitro* casting has been generally used to observe the course and morphological characteristics of aqueous veins. *In vivo* studies on the aqueous veins 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 photographs of the slit lamp microscope. 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[10-11]. However, HVI is a technology based on slit lamp images, and although the HVI software is modern and well designed, slit lamp imaging technology limits the accuracy of the measurements. The OCT manifestation of the aqueous vein can help to identify the aqueous vein. In the present study, OCT was used to objectively observe the aqueous vein. Multiple morphological characteristics were accurately measured and compared with those of the conjunctival vein, which helped to accurately identify aqueous veins according to these parameters. In addition, these parameters provided biological indicators for the evaluation of aqueous vein changes under different interventions.

OCTA is an emerging technology for imaging the 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 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[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. We found that the blood flow 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 agree with the anatomical and physiological characteristics of the aqueous vein and conjunctival vein, confirming the reliability of OCTA to observe the blood flow of the aqueous vein.

After the water-drinking test, the IOP increased, reaching a peak 15min after water loading, and it then gradually decreased and returned to the normal level 60min after water loading, which was consistent with earlier results[19-21]. Our results showed that the area of the aqueous vein decreased after the water-drinking test, reaching its nadir 15min after water loading, followed by a gradual increase to a normal level at 60min. A previous study showed that the aqueous humor flow was minimized approximately 10min after the water-drinking test[21]. This low flow may have directly led to the lowest aqueous vein area 15min after water loading, and then IOP increased. On the other hand, in our previous study, we reported that parasympathetic nervous system activity increased significantly after water loading[19], and whether this parasympathetic activation could lead to morphologic changes in the aqueous vein needs to be further explored. In addition, our results showed that the aqueous vein density decreased after the water-drinking test, which may have been 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 backscattered light[22]. Quantification of different reflective bands of OCT images has been widely used in retinal diseases and cataracts[23-24], but no OCT reflectivity of the aqueous vein has been reported. In our study, the aqueous vein was observed as a rounded, uneven gray black space on the OCT images. The grayscale value of the aqueous vein was higher than that of the conjunctival vein, and the OCT reflectivity of the aqueous vein was much lower, which may have been due to more water in the aqueous vein than in the conjunctival vein. This new information will be valuable 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 the 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[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, a distribution consistent with previous reports. Our study confirms that the aqueous vein in the inferior quadrant is more obvious and can 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, but 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 to study 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 aqueous vein variables after the water-drinking test.

In summary, our study objectively observed 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 provides a potential new method to evaluate the pathophysiology of glaucoma patients.

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Authors' contributions: Chen ZQ, Chen W, Deng CH, and Guo JM performed the research and data analysis, Chen ZQ and Chen W wrote the main manuscript text, Zhang H and Wang JM designed the study and revised the manuscript. All authors reviewed the manuscript.

Conflicts of Interest: Chen ZQ, None; **Chen W,** None; **Deng CH,** None; **Guo JM,** None; **Zhang H,** None; **Wang JM,** None.

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