In vivo Noninvasive Imaging and Quantitative Analysis of Iris Vessels

Wenwen Xue^a Senlin Lin^a Xia Chen^a Yanwen Jia^b Xiaoling Fang^a
Yan Suo^a Yingyan Ma^b Yulan Wang^{a, b} Haidong Zou^{a, b}

^aDepartment of Ophthalmology, Shanghai Eye Disease Prevention & Treatment Center/Shanghai Eye Hospital, Shanghai, China; ^bDepartment of Ophthalmology, Shanghai General Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China

Keywords

Quantitative analysis · Iris vessels · In vivo noninvasive imaging

Abstract

Objective: To quantify the iris vessels and its circadian rhythm in normal eyes. **Methods:** Fifteen healthy subjects were enrolled in this retrospective, cross-sectional study. All subjects underwent optical coherence tomography angiography (OCTA) and indocyanine green angiography (ICGA) examinations, in which 3/15 completed ICGA and OCTA at the same visit. Upon visit, consecutive OCTA scans were then obtained at the time points of the hour 3:00, 6:00, 8:00, 10:00, 12:00, 14:00, 16:00, 18:00, 20:00, 22:00, and 24:00, respectively. Vessel area density (VAD) and vessel skeleton density (VSD) were used to quantitatively describe the OCTA images of the iris vessels. **Results:** The VAD and VSD of the iris vessels had circadian rhythm with the highest values observed at about 18:00 h and lowest at 0:00 h; the overall values were relatively stable within the 24 h. The contour analysis suggested that the iris VAD and VSD were correlated with the changes in blood pressure and inversely correlated

with the changes in the intraocular pressure. **Conclusions:** OCTA can be used accurately for quantitative analysis of the iris vessels.

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Introduction

The human iris is an important and highly vascularized diaphragm that anatomically separates the anterior and posterior chambers of the eyeball [1]. Various characteristic or noncharacteristic lesions occur in the iris vasculature in the development of ocular abnormalities, degenerative diseases, retinal vascular obstruction, diabetic microvascular changes, glaucoma, uveitis, tumors, and surgery or accidental trauma, and the appearance of such lesions can often be used for efficacy evaluation and important clinical diagnosis [2]. For example, to determine the nature of iris tumors based on the local distribution of the iris blood vessels, the increase or decrease in iris neovascularization can

Wenwen Xue and Senlin Lin contributed equally to the manuscript.

be monitored and the severity of ocular ischemic lesions and the effect of treatment can be evaluated. However, only qualitative analyses have been conducted in previous studies, and no research report has accurately quantified the subtle changes in the iris vasculature largely due to the lack of suitable inspection techniques.

Human eyes have abundant pigment epithelium in the iris tissue, especially in the eyes of Asians and Africans. Slit lamp microscopes, ophthalmic ultrasound, and other inspection equipment cannot penetrate the pigment epithelium to observe the iris blood vessels. Indocyanine green (ICG), the contrast agent used in ICG angiography (ICGA), can absorb and emit near-infrared light and is less affected by the iris pigment. Therefore, ICGA has been used to display the characteristics of iris blood vessels and hemodynamics [3]. However, ICG flows and metabolizes rapidly in the iris blood vessels. Using the ICGA images, only qualitative analysis of iris blood vessels can be performed at a specific time during the arterial, capillary, or venous phase. Therefore, it is necessary to ensure highly consistent observation time points, in order to accurately compare minute changes in iris blood vessels before and after the occurrence and development of a disease or treatment. It is difficult to ensure such consistency in the actual operation. Moreover, ICGA is an invasive examination, and some patients may experience various degrees of allergic reactions or side effects [4].

In recent years, there have been rapid developments in the optical coherence tomography angiography (OCTA) technology. Through continuous B-scan detection of motion contrast of red blood cells, OCTA can reveal functional blood vessels permeating the living tissue. It has been widely used to observe a variety of eye-related microvascular diseases, both qualitatively and quantitatively [5, 6]. In terms of iris vascular observation, in 2014, Skalet et al. [7] used OCTA technology for the first time, to observe iris melanoma. Chien JL et al. [8] observed 4 cases of iris cirroid angioma and found that radial iris vessels that are not obvious on the fluoroscopic images are clearly displayed on the OCTA image. Unlike the ICGA examination, OCTA scans are not affected by the time of detection. Therefore, the iris vessel structure on OCTA images can be quantitatively analyzed and used to compare the development of diseases or the minute changes in the iris vessels before or after treatment. To this end, we exploratorily acquired the iris OCTA images of the human eye using the Cirrus 5000-HD-OCT (Carl Zeiss Meditec, Inc., Dublin, CA, USA) system equipped with angioplex blood flow imaging function. We believe that the results may provide a reference for the future investigations on using

the noninvasive OCTA technology and quantitatively observing the changes in the iris blood vessels during the occurrence and development of iris diseases as well as for the pre- and posttreatment assessments, such as DR, AMD, and other ischemic diseases.

Materials and Methods

This was a cross-sectional observational study conducted from January through March 2019 at the Shanghai Eye Disease Prevention & Treatment Center. The registration number of this study on ClinicalTrials.gov is NCT03631108. The study was approved by the Ethics Committee of the Shanghai General Hospital, Shanghai Jiao Tong University, Shanghai, China (Approval No. 2018KY181). Written informed consent was obtained from all participants.

Healthy volunteers were enrolled. The inclusion criteria were (1) ≥ 11 years and ≤ 60 years old, Han ethnicity, and Chinese nationality; (2) the spherical equivalent myopia of either eye was < -5 D. The exclusion criteria were (1) previous history of ocular trauma and eye surgery and other ocular diseases, (2) history of autoimmune diseases or receiving immunosuppressive therapy, (3) participated in clinical trials of intraocular or systemic drugs in the past 6 months, and (4) the OCTA image of the iris was not clear enough for further quantification and analysis.

Two technicians completed all the eye examinations. The presenting vision [9] and spherical equivalent lens power were measured [10]. Cirrus HD-OCT 5000 angiography system was used in the investigation. An external optical attachment was used in the system to enable the collection of iris OCTA scans (Fig. 1a), and 3×3 mm scan mode was applied. If the image clearly showed the iris vessels (from the edge of the pupil to the root of the iris) and some of the limbal vessels, the scanned image was considered good. For some healthy volunteers who had a small eyelid fissure, it was difficult to obtain blood vessels of the upper, lower, and nasal iris tissues. Therefore, we used the OCTA image of the temporal median iris as the candidate image for quantitative analysis.

In this study, 3 healthy volunteers were randomly selected for additional ICGA examination using a fluorescence machine (Heidelberg SPECTRALIS HRA, Germany). The arterial phase images were selected, and then the template matching method was used for image registration of ICGA and OCTA images. The specific details of this method are as follows [11, 12]: first, the ICGA (Fig. 1c) and OCTA images (Fig. 1b) of the same eye were matched. Then, the iris vessels were extracted from Figure 1b, and a template matching algorithm was used to check whether there was a corresponding result in Figure 1c. Second, using the global method of image traversal, the distribution characteristics of the pixel feature values of the 2 images were presented in the form of histograms. The larger the span of the features, the greater the amount of information about the features contained in the images (Fig. 1e).

A custom quantification software with an interactive interface in MATLAB (R2017a; MathWorks Inc., Natick, MA, USA) was made specifically for OCTA iris images based on a previously published algorithm of retinal vasculature quantification [6]. In brief, OCTA iris images were read into the custom software, and then an annulus region of interest was chosen by specifying the center, inner, and outer edges of this annulus. Both the hessian filter and

Fig. 1. **a** Photos of the iris OCTA image acquisition scene, and the arrow shows the installed +20 D lens. **b** An OCTA image of the temporal iris of the right eye of a healthy volunteer (subject 1). The arrow shows the observed iris vessels. On the OCTA images, we were able to clearly observe the small ring of iris arteries surrounding the pupil's edge, the large ring of iris arteries covered by the scleral margin tissue near the root of the iris, and the radial arrangement between the large ring and the small ring, mainly small arteries in the iris stroma layer. **c** An ICGA image of the temporal iris of the right eye of the same volunteer (subject 1). **d** A B-scan OCTA image of the temporal iris of the right eye of the same volunteer (subject 1). **e** Pixel distribution feature histogram of the OCTA image and the ICGA image of the temporal iris of the right eye of the same volunteer (subject 1) (black vertical stripes represent OCTA and black horizontal stripes represent ICGA). **f** An OCTA image of the temporal iris of the right eye of a neovascular glaucoma patient. **g** An ICGA image of the temporal iris of the right eye of the same neovascular glaucoma patient. OCTA, optical coherence tomography angiography; ICGA, indocyanine green angiography.

Fig. 2. Quantification of iris VAD and VSD. VAD, vessel area density; VSD, vessel skeleton density.

adaptive threshold were utilized to binarize the OCTA images, such that the pixels occupied by the blood flow were set to 1, otherwise 0. After acquiring the binary vessel map, a skeleton vessel map was generated by reducing the width of vessels in the binary vessel map to 1 pixel. Two quantitative metrics were calculated based on these 2 vessel maps, the iris vessel area density (VAD) and vessel skeleton density (VSD). Three consecutive measurements were taken and then averaged.

This study also examined the repeatability of OCTA quantitative metrics of the iris vasculature in healthy subjects. The inter-grader repeatability was assessed by 2 technicians scanning the same eye of the same volunteer 5 min apart, and the intervisit repeatability was assessed by the same technician scanning the same volunteer 24 h apart. The repeatability of VAD or VSD values was calculated using the intraclass correlation coefficient (ICC) values. We also performed a correlation analysis on the binocular iris OCTA quantitative index values of the same subject. The same technicians successively collected OCTA images of the iris area of the left and right eyes of the same volunteer, obtained the VAD and VSD values, and calculated the ICC values.

The process of analyzing circadian rhythm changes and related factors of healthy subject's iris OCTA quantitative indices was as follows: the same technician completed a total of consecutive 11 inspections in each healthy subject within the 24-h visit period: at the hour of 3:00, 6:00, 8:00, 10:00, 12:00, 14:00, 16:00, 18:00, 20:00,

22:00, and 24:00, respectively. We assumed that VAD/VSD of iris vessels was related with intraocular pressure (IOP). The time points for analyzing the healthy volunteers were set according to 24-h IOP measurements. At each time point, the blood pressure and IOP were measured. The temporal region of the right eye was used for circadian rhythm analysis. VAD and VSD curves were plotted, and the Friedman test was performed to statistically analyze the relationships among VAD, VSD, and the time of inspection.

We calculated the loop ratio for the measured values (VAD, VSD, blood pressure [mean arterial pressure [diastolic pressure $+1/3 \times$ pulse pressure difference], and IOP) at each time point. The calculation formula was: $\text{rate} = (\text{value}_t - \text{value}_{t-1}) / \text{value}_{t-1} \times 100\%$, where "value_t" represents the index value (of VAD, VSD, blood pressure, or IOP) measured at the time "t" and "value_{t-1}" represents the index value at a point before time "t." The calculated loop ratios were plotted and profiled for statistical analysis to investigate the relationship of the changes in VAD and VSD with blood pressure and IOP.

The sample size calculation for the analysis of circadian rhythm changes in this study applied the bioequivalence in the reference of drug experiment, and the calculated sample size was 7 people. The above statistics were analyzed using SAS 9.4 (SAS Institute, Inc., Cary, NC, USA).

Table 1. VAD and VSD values on the temporal side of right eyes, blood pressure, and IOP of 15 healthy volunteers in 24 h

Time point	VAD	VSD	NCT, mm Hg	Blood pressure, mm Hg
8:00	0.21±0.02	0.16±0.02	15.53±1.97	82.31±12.67
10:00	0.22±0.03	0.16±0.02	15.21±2.32	83.56±11.43
12:00	0.21±0.03	0.16±0.03	14.94±1.98	82.76±8.83
14:00	0.22±0.04	0.15±0.03	14.75±2.31	80.93±10.27
16:00	0.21±0.03	0.15±0.02	15.25±3.03	82.56±13.79
18:00	0.21±0.04	0.15±0.02	14.71±2.74	85.96±10.85
20:00	0.21±0.03	0.15±0.02	13.93±2.37	84.58±13.91
22:00	0.21±0.02	0.15±0.01	14.27±2.12	85.29±12.38
0:00	0.20±0.04	0.15±0.03	14.80±2.78	83.53±11.68
3:00	0.22±0.04	0.16±0.02	15.37±3.29	85.67±14.22
6:00	0.21±0.04	0.15±0.02	16.03±3.55	80.69±12.02

VAD, vessel area density; VSD, vessel skeleton density; IOP, intraocular pressure.

Results

A total of 40 healthy subjects were included in this study. Fifteen iris OCTA images were of good quality, and the other 25 subjects were excluded from the study because their iris OCTA blood vessel images were unclear (because OCTA signal did not penetrate through heavy pigments in 18 subjects, severe motion artifacts in 5 subjects, and low signal strength index in 2 subjects).

The 3 healthy subjects included in this part of the study were young men (mean age, 29 years). ICGA and OCTA images were all successfully acquired. The image registration results were as follows: first, all the vessel images on the ICGA map were detected in the corresponding areas of the OCTA map, indicating that all the vascular features identified using the ICGA image were included in the OCTA image. Second, the OCTA and ICGA distribution feature histograms (Fig. 1e) showed that the OCTA image feature distribution was more extensive than that of the ICGA image, indicating that OCTA contained more feature information than ICGA (horizontal axis: pixel value feature; vertical axis: feature distribution). Moreover, some of the radial iris vessels (shown by arrows) displayed by the iris OCTA were not obvious on the ICGA image. In Figure 1d, when compared with the corresponding B-scan image, a clear red blood flow signal can be seen. We also examined neovascular glaucoma patients and found iris neovascularization can be shown with iris OCTA, as clear as ICGA (Fig. 1f, g).

The obtained OCTA data were then analyzed to obtain the vessel parameters of VAD and VSD (Fig. 2). The same technician performed repeated measurements on the same subject at the same time point (e.g., at the hour of 08:00 on the first and the second day) for the test of intervisit repeatability (VAD, ICC = 0.946, 95% CI: 0.885–0.98; VSD, ICC = 0.908, 95% CI: 0.764–0.967). Two technicians performed repeated measurements on the same subject at 5-min intervals on the same day for intergrader repeatability (VAD, ICC = 0.947, 95% CI: 0.857–0.981; VSD, ICC = 0.944, 95% CI: 0.849–0.98). The results of the left and right eyes of the same subject were tested for correlation at the same time, on the same day (VAD, ICC = 0.981, 95% CI: 0.924–0.995; VSD, ICC = 0.875, 95% CI: 0.576–0.967).

Table 1 shows the VAD and VSD values on the temporal side of right eyes, blood pressure, and IOP of 15 healthy volunteers at 11 time points in 24 h. The results showed that the highest values of VAD and VSD appeared at 18:00 in the afternoon and the lowest value appeared at midnight.

We used contour analysis to explore the relationship of VAD and VSD with blood pressure and IOP (Fig. 3). The overall contours of VAD and blood pressure and IOP were parallel to each other (Hotelling's Trace, $p = 0.732, 0.310$) and coincided ($p = 0.196, 0.988$). Therefore, the VAD can be considered consistent with daily changes in blood pressure and IOP. Similarly, the overall contours of VSD and blood pressure and IOP were parallel to each other (Hotelling's Trace, $p = 0.704, 0.504$) and coincided ($p = 0.404, 0.767$). It can be considered that the VSD was consistent with the daily changes in blood pressure and IOP.

Fig. 3. Contour analysis of the VAD/VSD, blood pressure, and IOP. VAD, vessel area density; VSD, vessel skeleton density; IOP, intraocular pressure.

Discussion

The rationale behind the inclusion and exclusion criteria was as follows: (1) considering that all kinds of eye diseases may be accompanied by iris vascular disease, they may affect the analysis of circadian rhythm changes; (2) autoimmune diseases are often associated with uveitis; (3) young children may have difficulty in cooperating with OCTA examinations, and elder people over 60 years of age often have cornea arcus senilis, which affects the observation of iris vessels; and (4) highly myopic eyes generally have a deeper anterior chamber, which may cause distortion of the iris vessel imaging map.

In addition to the arterial iris vessels observed under ICGA, OCTA clearly observed more radial iris vessels that were not obvious on ICGA images. Through image registration, we found that the OCTA images contained more vascular features than the ICGA images, and some vessels that were visible on the OCTA images were not

detectable on the ICGA images. However, clearer blood flow signals were seen on the corresponding B-scan image (Fig. 1d). A possible explanation is that OCTA can detect the venules in the deeper layer of the iris that were not visible on the arterial phase of ICGA. Therefore, we believe that more iris vascular information can be obtained by using the OCTA images than the arterial-phase ICGA images.

The OMAG algorithm uses moving particles (such as red blood cells) to highlight the flow signals against static tissue signals and has better vascular connectivity, higher signal-to-noise ratio, and is more sensitive to capillary blood flows than the other microvascular imaging technologies [13–15]. In this study, we provided quantitative measures of the OMAG images to obtain the VAD and VSD values of iris vessels. The VAD is calculated as a unitless ratio of the total image area occupied by the vasculature to the total image area in the binary vessel maps. The VSD is calculated as the ratio of the length occupied

by the blood vessels to the total area in the skeletonized vessel map. The combination of VAD and VSD could provide a more accurate and detailed morphological information of the iris vessels. The ICC values in this study were all >0.75 , suggesting good consistency and repeatability for VAD and VSD indices. This not only confirmed our speculation that the iris vascular structure is stable in the healthy human eyes or in the stable period of eye diseases but also indicated that these 2 quantitative indices could be used for accurate quantitative analysis of iris vascular changes in the human eyes. This study also found that the VAD and VSD values were highly consistent in the left and right eyes of the same volunteer, which further confirmed the reliability of the 2 iris blood vessel quantitative indices [16]. Furthermore, it provided a scientific basis for us to use the right eye data to represent an individual's iris vascular VAD and VSD values.

Previous studies have shown that circadian rhythms exist in many physiological parameters of the eyes, such as the IOP, choroidal blood flow, and retinal thickness. We found that VAD and VSD values of iris vessels in a normal human eye also had circadian rhythm, with the highest value at about 18:00 and lowest at midnight; overall, the values were relatively stable within the 24-h observation window. The circadian rhythm plays an important role in adapting to the body's activities and protecting the heart and brain blood vessels. In physiological state, iris vessels have the ability to regulate themselves automatically, that is, when the perfusion pressure changes within a certain range, the blood vessels contract or relax through the transmission between adjacent smooth muscle cells, in order to maintain the stability of the local blood flow [17]. In our opinion, this is why VAD and VSD values were essentially stable during the 24-h continuous observation period.

In this study, contour analysis suggested that the iris VAD/VSD was consistent with changes in the blood pressure and was inversely related to the changes in IOP. Similar to our results, Mottet et al. [18] monitored the changes in IOP for 24 h and found that the IOP of normal people had obvious circadian rhythms with the highest value at 3:00 and lowest at about 15:00. Blood pressure also had an obvious circadian rhythm. Generally, there were 2 peaks at 6:00–10:00 a.m. and 4:00–8:00 p.m., and at night, the blood pressure was significantly reduced, with the lowest level observed at 0:00–3:00 a.m. [19, 20]. Duke-Elder [21] noticed that changes of IOP with changes of arterial blood pressure may have an opposite relationship. Our study provides further evidence for the relationship among IOP, blood pressure, and circadian

rhythm of the iris blood flow, suggesting that there is a certain internal connection among them. A possible reason may be that the perfusion pressure of a tissue or organ is the driving force to maintain its blood flow supply. Only with sufficient perfusion pressure can the blood flow meet the needs of iris function. This process needs to be regulated by the pressure difference between the arteries and the veins. Tissue or organ perfusion pressure is equal to the difference between the arterial and venous pressure. In the eyes, the IOP can be considered equal to the venous pressure. Generally, the average perfusion pressure of the eyes is calculated by the difference between the average arterial pressure and the IOP [22]. Therefore, the VAD/VSD value of the iris is consistent with the change in blood pressure and opposite to the change in IOP.

The clinical application of any new technology has its limitations. The disadvantages of using OCTA technology to analyze iris vessels are as follows: (1) like other optical and acoustic examinations, the OCTA examination is affected by the target tissue or organ. Due to pigment occlusion and tissue thickness, iris vessels were not observed fully in many volunteers on the OCTA images. Skalet et al. [7] reported similar factors in their study on iris melanoma. Pigment occlusion is an unavoidable disadvantage, and OCTA is currently known as the method that can obtain the most iris vascular information. (2) We can only get the image of the shallow iris vessels, but not the deep ones. Perhaps a longer wavelength OCTA system can overcome this defect. (3) Since the iris tissue is thicker in the periphery, the peripheral iris vascular imaging was not as clear as the pupil margins. Therefore, there may be some deviations when selecting different parts of the iris for quantitative blood vessel analysis. (4) OCTA determines the blood flow by detecting the movement of red blood cells in blood vessels, so the movement of the eyeball during the image acquisition may cause artefacts. In the process of iris OCTA examination, poor fixation, nystagmus, or poor vision may also cause poor imaging quality due to the influence of eye movement. (5) We only included normal subjects in this study, and future studies are warranted to establish the differences between normal and abnormal iris vasculature with OCTA. (6) Only Han ethnicity was included in this study, and future studies are needed to image the iris vessels of different ethnicities, different genders, and different age groups. Furthermore, the included volunteers were all emmetropia or had low myopia. Therefore, multicenter, large-scale prospective studies are needed in the future.

Conclusion

After searching the PubMed database, we believe that our team has completed the first study on quantitative index analysis of iris vessels. In conclusion, our results can be used for accurate quantitative analysis of iris lesions. It is expected to be used for auxiliary diagnosis and efficacy evaluation of various iris diseases or other iris complications. It can also be used to explore the mechanism of iris vascular disease in vivo, in the future.

Statement of Ethics

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the Declaration of Helsinki. Written informed consent was obtained from all individual participants included in the study. The study was approved by the Ethics Committee of the Shanghai General Hospital, Shanghai Jiao Tong University, Shanghai, China (Approval No. 2018KY181).

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

W.W.X. and S.L.L. are joint first authors. W.W.X., S.L.L., and H.D.Z. analyzed the data and drafted the manuscript. Y.L.W. and Y.Y.M. contributed to the critical revision of the manuscript. X.C., X.L.F., S.Y., and Y.W.J. collected the data. L.G. designed the study. All authors have read and approved the final manuscript.

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