Distinctive Analysis of Macular Superficial Capillaries and Large Vessels Using Optical Coherence Tomographic Angiography in Healthy and Diabetic Eyes

Jianqin Lei,1 Enhui Yi,2 Yan Suo,3 Cheng Chen,3 Xiayu Xu,4,5 Wenxiang Ding,4,5 Nizar Saleh Abdelfattah,6,7 Xiaojuan Fan,1 and Huiqin Lu3

1Department of Ophthalmology, First Affiliated Hospital of Xi’an Jiaotong University, Xi’an, China
2Department of Ophthalmology, Xi’an Central Hospital, Xi’an, China
3Department of Ophthalmology, Xi’an No. 1 Hospital, Xi’an, China
4The Key Laboratory of Biomedical Information Engineering of Ministry of Education, School of Life Science and Technology, Xi’an Jiaotong University, Xi’an, China
5Bioinspired Engineering and Biomechanics Center (BEBC), Xi’an Jiaotong University, Xi’an, China
6Doheny Image Reading Center, Doheny Eye Institute, Los Angeles, California, United States
7Department of Ophthalmology, David Geffen School of Medicine at UCLA, Los Angeles, California, United States

PURPOSE. To quantify and evaluate macular superficial capillaries and large vessels separately using an optical coherence tomographic angiography (OCTA)-based automatic segmentation algorithm.

METHODS. In this cross-sectional study, all eyes were scanned using an OCTA device with 3 × 3 mm cube centered on the fovea. Retinal large vessels (arterioles/venules) were automatically segmented from superficial vasculature en-face images. All images were normalized, binarized, and skeletonized for quantification. Metrics of retinal capillaries were calculated by subtracting the measurements of large vessels from total vasculature. Perfusion density (PD), vessel length density (VLD), and vessel diameter index (VDI) within Early Treatment Diabetic Retinopathy Study (ETDRS) 3-mm ring were calculated for total superficial vasculature, large vessels (PDlarge, VLDlarge, and VDIlarge) and capillaries (PDcap, VLDcap, and VDIlcap), respectively.

RESULTS. Fifty-nine eyes from 59 healthy participants (mean age, 45 ± 14 years, 36 females) and 118 eyes from 67 patients with diabetes mellitus (mean age, 57 ± 10 years, 28 females) were included. The diabetic cohort included four subgroups (35 eyes without diabetic retinopathy, 30 eyes with mild to moderate nonproliferative diabetic retinopathy [NPDR], 27 eyes with severe NPDR, and 26 eyes with PDR). Linear regression showed that all above metrics were correlated with the disease stage (from healthy state to PDR), and the β value was −0.76, 0.24, −0.78, 0.80, 0.30, 0.77, −0.81, 0.16, and −0.82 for VD, VDlarge, VDcap, VDI, VDIlarge, VDIlarge, VLDlarge, and VLDcap, respectively.

CONCLUSIONS. Retinal capillaries and large vessels responded differently in the context of diabetes. VLD of capillary is a potentially reliable metric in diabetic retinopathy staging.

Keywords: optical coherence tomography angiography, diabetic retinopathy, macular superficial capillaries, segmentation, measurement

Optical coherence tomographic angiography (OCTA) is a recently developed imaging modality that can facilitate noninvasive study of retinal or choroidal vasculature in details.1 It compares the variation between consecutive B scans that reveals the motion of erythrocyte. Due to its high resolution, retinal microvasculature can be displayed on separate layers and quantitative metrics can be achieved by automated image analysis.

Diabetic retinopathy is the most common microvascular complication of diabetes.2 Many studies revealed that OCTA was useful in the evaluation of retinal ischemia and capillary network.3,4 Also, quantitative metrics such as vessel density, skeletonized density, vessel diameter index (VDI), fractal dimension, nonperfusion area, and area/circularity of foveal avascular zone (FAZ) have been used to measure the retinal vasculature from diabetic retinopathy.5−7

Previous measurements of retinal superficial capillary plexus (SCP) included both capillaries and larger vessels (arterioles and venules). It is known that the retinal arterioles contain smooth muscle cells while the capillary wall is composed of pericytes and endothelial cells. Though precisely what modulates blood flow dysregulation in arterioles and capillaries during diabetic retinopathy remains uncertain,8 it has been well established that pericytes loss from apoptosis can occur at a very early stage of diabetic retinopathy.9 Thus, the hemodynamic and structural change from hyperglycemia for capillaries and larger vessels may not exactly be the same. Morphological changes in the retinal arterioles and venules have previously been reported to offer prognostic values for the prediction of diabetic
retinopathy. However, very few reports focused on retinal capillaries specifically in vivo because of the limited resolution by traditional modalities. Based on the optical micro-angiography algorithm of OCTA, the intensity of the blood flow is proportional to the amount of red blood cells passing through the vessels. Thus, retinal larger vessels should have a higher intensity compared to capillaries. Based on different diameter and intensity, it is possible for us to develop an algorithm that can distinguish the two elements of retinal vessels. In this cross-sectional study, we used a method described by the laboratory from life science and technology school of Xi’an Jiaotong University to segment the larger vessels from retinal SCP and measured the retinal capillaries and larger vessels separately in healthy and diabetic eyes. Our purpose is to find out whether the two elements of retinal vessels act differently in the context of diabetes.

METHODS

Data Collection

In this cross-sectional single-center study, two cohorts were enrolled at Xi’an No. 1 Hospital from February to June 2017. One cohort included healthy volunteers over 20 years old without any history of eye diseases. They were further divided into three age groups (group 1, age 20–40; group 2, age 40–60; group 3, age ≥ 60), with approximately 20 participants in each group. One eye was randomly chosen from each participant to be imaged with 3 × 3 mm scan pattern centered on the fovea (AngioPlex, software version 9.0, Cirrus HD-5000, Carl Zeiss Meditec, Dublin, CA, USA). The scan was repeated on the same eye immediately after repositioning the participant. Another cohort enrolled patients diagnosed with diabetes with or without diabetic retinopathy. Both eyes of every patient were imaged with 3 × 3 mm scan pattern on the same device. At least one acquisition was achieved for each eye. The diabetic cohort included four subgroups: (1) eyes without diabetic retinopathy (wDR group); (2) mild to moderate nonproliferative diabetic retinopathy (mNPDR group); (3) severe NPDR (sNPDR group); and (4) proliferative diabetic retinopathy (PDR group). All diagnoses were made by two medical retina specialists (H.L. and Y.S.). Eyes were excluded from the study if there was evidence of optical media opacity, maculopathy caused by other disease, or refractive error ≤ –6D or ≥ 3D. Exclusion criteria related to the images included signal strength (SS) <7, distinct motion artifact (more than two lines of saccades), macular deviation from center, or ”wrong segmentation” of the large vessels (described below). Written informed consent was obtained from all individuals prior to study participation. The study was approved by the Institutional Review Board of Xi’an Jiaotong University and conducted in accordance with the ethical standards stated in the Declaration of Helsinki.

Image Processing

En-face images of SCP (automatically segmented from internal limiting membrane to defined inner plexiform layer) were generated by the AngioPlex software (version 9.0). Normalization of the images was based on the internal intensity of FAZ. Three circles (100 pixels in diameter) were placed in different locations within the FAZ (Fig. 1A). Averaged intensity within each circle was calculated and the normalized image was obtained after subtracting the mean value of the three circles from the whole image (Fig. 1B). Hessian filter was used to enhance all the microvasculature in the normalized images, followed by a background denoising using an automatic thresholding (Fig. 1C). Then, the binarized vessel images were further skeletonized to obtain the single lines of the vessels (Fig. 1D).

Retinal large vessels (arterioles and venules) were defined as the arcades and their first and second branches. Automatic segmentation of these large vessels was achieved using a multiscale line detector. Three different scales (27 × 27, 37 × 37, and 47 × 47 pixels) were used in this method. In each scale, for a pixel (x,y) in the normalized image I, eight line detectors centered at (x,y) with equal angle increment ranging from 0° to 180° were generated and convolved with the normalized image and the maximum response was recorded as $C^m_{ini}(x,y)$. The mean value of the normalized image patch at scale $n_i$ before convolution was defined as $I^m_{ini}(x,y)$. The output of the line detector at point (x,y) was defined as $C^m(x,y) = C^m_{ini}(x,y) - I^m_{ini}(x,y)$. The final output image with retinal arterioles and venules enhanced was defined as $O = \sum_{n_i} C^m_{ini} + I$, followed by thresholding and denoising to obtain the binarized image (Fig. 1E). An example of corresponding skeletonized image is shown in Figure 1F. “Wrong segmentation” was defined as over 30% of the first branches from the arcade vessels undiscovered. “Excellent segmentation” was defined as less than 10% of the first branches undiscovered.

The image processing and program making was based on the software Visual Studio 2010 (Microsoft, Redmond, WA, USA).

OUTCOME MEASURES

We measured the perfusion density (PD), vessel length density (VLD), and VDI within 3-mm ring defined by Early Treatment Diabetic Retinopathy Study (ETDRS) on retinal SCP. PD was calculated as the percentage of the white pixels from the total pixels in the target area based on the binarized image (Fig. 1C). In Equation 1, $v(x,y)$ represents a pixel on the segmented vessel within central 3-mm ring, and $T(x,y)$ represents any pixel in the central 3-mm ring.

$$PD = \frac{\sum_{(x,y)} v(x,y)}{\sum_{(x,y)} T(x,y)} \quad (1)$$

VLD is the vessel length per unit area calculated based on the skeletonized image (Fig. 1D). The equation is as follows:

$$VLD(\text{mm}^{-1}) = \frac{\sum_{(x,y)} s(x,y) T(x,y)}{\sum_{(x,y)} T(x,y)} \times \frac{N}{L} \quad (2)$$

$s(x,y)$ represents a pixel of vessel within 3-mm ring based on skeletonized image, $L$ is the length of the scanned area, and $N$ is the corresponding number of pixels ($L$ is 3 mm and $N$ is 1024 in this study). VDI is defined as the area of vessels divided by their length. Therefore, it represents the approximate vascular width. The calculation of VDI is as follows:

$$VDI(\text{mm}) = \frac{PD}{VLD} \quad (3)$$

The vessel density of large vessels was calculated from images (Figs. 1E, 1F) and was termed as $PDLarge$ and $VLD_{large}$. Vessel density of superficial capillaries was labeled as $PD_{cap}$ and $VLD_{cap}$.

$$PD_{cap} = PD - PDLarge; \quad VLD_{cap} = VLD - VLD_{large} \quad (4)$$

$VDI_{large}$ and $VDI_{cap}$ were both calculated using Equation 3.
Statistical Analysis

All the statistics were calculated using software SPSS 23.0 (IBM Corporation, Armonk, North Castle, NY, USA). Intraclass correlation coefficients (ICCs) and coefficient of variance (CV) were calculated between the initial and secondary acquisitions to test the repeatability of outcome measures on the same visit. We also assessed factors that could affect measurement variability in healthy cohort. First, we compared SS, PD, VLD, VDI, PD_cap, VLD_cap, VDI_cap, PD_large, VLD_large, and
Repeatability of Metrics

Within the healthy cohort, the ICC between two consecutive scans for PD, VLD, PDcap, and VLDcap was 0.83 (95% confidence interval [CI]: 0.71–0.90), 0.79 (95% CI: 0.65–0.88), 0.88 (95% CI: 0.80–0.93), and 0.81 (95% CI: 0.68–0.89), respectively. The CVs of those metrics were 1.6% ± 1.3%, 2.8% ± 2.5%, 1.9% ± 1.6%, and 3.1% ± 2.7%, respectively.

Factors Potentially Affecting Measurements

Variability

In the healthy cohort, there was no significant difference between genders for the measurements of total vasculature and capillaries. However, measurements for large vessels were higher in males compared with females. The mean difference between males and females was 1% for PDlarge (P = 0.001), 0.3 mm–1 for VLDlarge (P = 0.002), and 0.6 μm for VDIlarge (P = 0.037).

The measurements of different vessels by different age groups are displayed in Table 1. There were significant differences among age groups for PD (P = 0.042), VLD (P = 0.033), VDI (P = 0.053), and VDIcap (P = 0.004). Stepwise linear regression showed that only SS remained in the model and was the predictor for measurement of PD (β = 0.58), VLD (β = 0.55), VDI (β = −0.46), PDcap (β = −0.57), and VLDcap (β = −0.56), while age was the model predictor for VDIcap (β = 0.45).

Neither age nor SS was correlated with measurements on large vessels.

Healthy Versus Diabetic Groups Measurements

The mean values of age, SS, PD, VLD, and VDI of various types of vessels by different groups are displayed in Table 2. There was significant difference among the groups for all the above metrics. Regression analysis showed that the stage of diabetic retinopathy predicted change of all metrics, and it turned out that the regression coefficient (β) for VLDcap was closest to 1(–1). The value of β was −0.76, 0.24, −0.78, 0.80, 0.30, 0.77, −0.81, 0.16, and −0.82 for PD, PDlarge, PDcap, VDI, VDIlarge, VLDcap, VLDlarge, and VDIcap, respectively. Plots that describe the relationship between disease severity and each metric are displayed in Figure 2.

Compared to healthy group, the VD, VLD, VDIlarge, and VLDcap in wDR group were significantly lower, and VDI and VDIcap in this group were significantly higher. Their P values are all less than 0.001. The VLDlarge in wDR group was lower than that in healthy group with a marginal significance (P = 0.050) while there was no difference in PDlarge (P = 0.205) and VDI and VDIcap (P = 0.089) between the two groups. The age was higher (P < 0.001) in wDR group, while SS did not differ between the two groups (P = 0.08).

Sensitivity and Specificity in Detection of Mild to Moderate NPDR

The area under ROC curve was 0.938 (95% CI: 0.890–0.986), 0.952 (95% CI: 0.910–0.994), 0.946 (95% CI: 0.904–0.989), 0.937 (95% CI: 0.887–0.987), 0.955 (95% CI: 0.915–0.996), and
### TABLE 2. The Mean Values of Measurement on Retinal Superficial Vasculature Within ETDRS Inner Ring From Healthy and Diabetic Eyes and a Comparison Among All Different Groups

<table>
<thead>
<tr>
<th>Metrics</th>
<th>Healthy, n = 59</th>
<th>wDR, n = 35</th>
<th>mNPDR, n = 30</th>
<th>sNPDR, n = 27</th>
<th>PDR, n = 26</th>
<th>F Value (ANOVA)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SS</td>
<td>9.7 ± 0.7</td>
<td>9.5 ± 0.7</td>
<td>9.0 ± 0.9</td>
<td>8.7 ± 1.0</td>
<td>8.0 ± 1.0</td>
<td>23.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age</td>
<td>45 ± 14</td>
<td>61 ± 9</td>
<td>58 ± 9</td>
<td>53 ± 11</td>
<td>54 ± 9</td>
<td>13.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PD, %</td>
<td>42.7 ± 1.7</td>
<td>40.6 ± 2.2</td>
<td>38.2 ± 3.2</td>
<td>36.0 ± 3.3</td>
<td>34.7 ± 3.3</td>
<td>59.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>VLD, mm⁻¹</td>
<td>24.3 ± 1.5</td>
<td>22.1 ± 1.7</td>
<td>20.3 ± 2.3</td>
<td>18.5 ± 2.2</td>
<td>17.2 ± 2.3</td>
<td>80.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>VDI, μm</td>
<td>17.4 ± 0.5</td>
<td>18.2 ± 0.6</td>
<td>18.7 ± 0.9</td>
<td>19.4 ± 0.7</td>
<td>20.0 ± 1.0</td>
<td>77.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PDlarge, %</td>
<td>7.5 ± 1.1</td>
<td>7.2 ± 1.1</td>
<td>7.4 ± 1.2</td>
<td>7.8 ± 1.3</td>
<td>8.6 ± 1.6</td>
<td>5.0</td>
<td>0.001</td>
</tr>
<tr>
<td>VLDlarge, mm⁻¹</td>
<td>3.2 ± 0.4</td>
<td>3.0 ± 0.4</td>
<td>3.1 ± 0.5</td>
<td>3.2 ± 0.5</td>
<td>3.4 ± 0.6</td>
<td>3.7</td>
<td>0.006</td>
</tr>
<tr>
<td>VDIlarge, μm</td>
<td>23.6 ± 1.0</td>
<td>23.9 ± 1.0</td>
<td>24.0 ± 1.2</td>
<td>24.3 ± 1.5</td>
<td>24.6 ± 1.4</td>
<td>4.4</td>
<td>0.002</td>
</tr>
<tr>
<td>PDcap, %</td>
<td>35.2 ± 1.9</td>
<td>33.3 ± 2.0</td>
<td>30.8 ± 3.0</td>
<td>28.3 ± 3.2</td>
<td>26.1 ± 3.8</td>
<td>67.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>VLDcap, mm⁻¹</td>
<td>21.1 ± 1.5</td>
<td>19.1 ± 1.7</td>
<td>17.2 ± 2.2</td>
<td>15.3 ± 2.1</td>
<td>13.7 ± 2.4</td>
<td>88.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>VDIcap, μm</td>
<td>16.5 ± 0.5</td>
<td>17.3 ± 0.6</td>
<td>17.8 ± 0.9</td>
<td>18.4 ± 0.8</td>
<td>18.9 ± 0.9</td>
<td>62.9</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

**FIGURE 2.** Estimation curve drawn from linear regression model for each metric. Group 0, healthy cohort. Group 1, eyes with diabetes but wDR. Group 2, eyes with mNPDR. Group 3, eyes with sNPDR. Group 4, eyes with proliferative diabetic retinopathy.
Measurement of Retinal Capillaries and Larger Vessels

0.938 (95% CI: 0.890–0.987) for PD, VLD, VDI, PD\textsubscript{cap}, VLD\textsubscript{cap}, and VDI\textsubscript{cap}, respectively. If the sensitivity is 90%, the specificity to detect mild to moderate NPDR for each metric will be 86%, 90%, 80%, 88%, 92%, and 83%, respectively.

**Evaluation of Large Vessel Segmentation**

Among all scans of healthy eyes (124 en-face images), none of the large vessel segmentation was graded as “wrong segmentation,” and 98.4% (122/124) of the images were graded as “excellent segmentation.” Among all the scans of patients with diabetes, 2.3% (3/129) of the images were graded as “wrong segmentation.” And 94.6% (122/129) of the images were graded as “excellent segmentation.” Some abnormally dilated vessels in the capillary bed can also be segmented as large vessels.

**DISCUSSION**

In this cross-sectional study, we evaluated retinal superficial vasculature in healthy and diabetic eyes using an automated method that can reliably segment retinal arterioles and venules from SCP. It turned out that retinal large vessels had a tendency of enlargement while the superficial capillaries dropped dramatically when the severity of diabetic retinopathy increased. The PD, VLD, and VDI of retinal superficial capillaries are useful metrics in evaluation of diabetic retinopathy, which turned out to be noninferior compared with previous parameters measuring the total superficial vasculature.

To our knowledge, this is the first study to measure retinal large vessels and capillaries separately using OCTA. The total vessel density of retinal superficial plexus and other metrics have been demonstrated in several studies to be correlated with the severity of diabetic retinopathy with relatively small sample sizes. Durbin et al. reported that the area under ROC curve to detect NPDR was 0.89 for VLD and 0.79 for PD. And by far, no consensus has been achieved on the staging of DR based on OCTA metrics. Thus, it is still worth exploring alternative approaches. We propose that VLD\textsubscript{cap} would be a promising metric for evaluation of diabetic retinopathy. The repeatability of VLD\textsubscript{cap} was high and it is less affected by age or gender variation. More importantly, it has a high sensitivity and specificity (area under ROC curve is 0.96) in detecting mild to moderate diabetic retinopathy since hyperglycemia initially affects the pericytes and endothelial cells of capillaries. Though the difference between capillaries and total vessels in evaluating diabetic retinopathy were not significant in our study, the larger vessels only made up approximately 10% to 20% of the total vessels. If the studied area had been larger, for example, the 6 × 6-mm macular area, or the scan had been centered on the optic disc, it might have made a difference.

Besides, retinal precapillary and postcapillary vessels can also be quantified using this method. We found that the diameter of large vessels steadily increased from diabetes without retinopathy to more severe stages of diabetic retinopathy, which could result from dilation of retinal venules and maybe the increase of retinal blood flow. This phenomenon complies with previous studies of retinal blood flow using bidirectional laser Doppler velocimetry. We suppose that the increased blood flow in larger vessels might result from a decreased resistance of capillaries and post-capillary vessels, since PD and VLD both dropped dramatically while the diameter index enlarged for retinal capillaries with increased severity of diabetic retinopathy. In late stages, vascular remodeling also accounted for the increasing of large vessels, since intraretinal microvascular abnormalities (IRMA) can be segmented as large vessels. Noticeably, the VLD\textsubscript{large} in diabetic eyes wDR was lower compared to the healthy group with a marginal significance ($P = 0.05$), with similar male to female ratio (14/21 in wDR group and 23/36 in healthy group) that could influence the measurement of large vessels. On the other hand, the VLD\textsubscript{large} in mNPDR and sNPDR group was similar to that of healthy group ($P = 0.317$ and $P = 0.912$, respectively). Therefore, we suspect that the fewer distribution of large vessels in diabetic patients could be a protective factor from diabetic retinopathy.

In diabetic eyes wDR, PD and VLD of superficial capillaries were significantly lower and the diameter of these vessels was significantly higher than those of healthy controls with similar SS in the two groups, while no significant difference was found for larger vessels between the two groups. This indicates that structural damage may happen initially on retinal capillaries before any clinical ocular manifestations take place in diabetic patients. Dimitrova et al. also proved that superficial and deep retinal vessel density in parafovea of diabetic patients wDR both decreased compared to healthy subjects. The demographics of the two groups were comparable in their study; however, they did not compare SS, which is a strong predictor for the measurements of vessel density.

In the healthy cohort, we found significant differences in PD and VLD among different age groups, as reported from the previous study using Angiovue. However, such difference was not significant for PD\textsubscript{cap} and VLD\textsubscript{cap}. Besides, we demonstrated that age was a positive predictor for VDI\textsubscript{cap} independent of SS. That may explain why PD did not drop much in older group, since the capillaries dilated with aging. One previous study has shown the strong correlation between SS and PD/VLD based on AngioPlex. In the present study, when both age and SS were put in the regression model, the PD, VLD, and VDI\textsubscript{large} were significantly lower and the diameter of these vessels was higher in males compared with females. And this might be explained by the androgen levels, which has been suggested to be associated with increased production of red blood cells.

There are several limitations for this study. First, the SS was not comparable among disease groups and healthy group, which could also contribute to the decrease of vessel density in the disease group. In real clinical practices, it is usually difficult to achieve the same image quality in eyes with severe diabetic retinopathy as that in healthy eyes. Still, we managed to ensure a SS of no less than 7 for all images. Second, the mean age and gender is also incomparable among the cohorts, but as we have demonstrated, age is less important, especially for the PD and VLD of capillaries, while gender could have affected the comparison for large vessels. Another limitation is that we could not distinguish arterioles from venules during the automatic segmentation of large vessels.

**CONCLUSIONS**

In summary, despite those limitations, this study still revealed that retinal larger vessels and capillaries acted differently in the context of diabetes using an OCTA-based quantified measurement. Retinal capillaries dropped significantly even in eyes without retinopathy for patients with diabetes. Among all analyzed metrics, VLD\textsubscript{cap} had the highest sensitivity and specificity in detecting mild to moderate diabetic retinopathy. Though not significantly, it is still a promising parameter in OCTA-based studies. Besides, SS can impact the measurement of retinal capillaries, but not on metrics of larger vessels.
Retinal capillaries become more dilated with aging, while retinal large vessels do not change significantly, with only gender affecting large vessels measurements.

Acknowledgments

Disclosure: J. Lei, None; E. Yi, None; Y. Suo, None; C. Chen, None; X. Xu, None; W. Ding, None; N.S. Abdelfattah, None; X. Fan, None; H. Lu, None

References


