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

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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 VDLarge) and capillaries (PDcap, VLDcap, and VLDcap), 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, VDLarge, VDLarge, and VDLarge, 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.
Measurement of Retinal Capillaries and Larger Vessels

**METHODS**

**Data Collection**

In this cross-sectional single-center study, two cohorts were enrolled at 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.

**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 ($27 \times 27$, $37 \times 37$, and $47 \times 47$ pixels) were used in this method. In each scale $n_i$, for a pixel $(x,y)$ in the normalized image $I$, eight line detectors centerd at $(x,y)$ with equal angle increment ranging from $0^\circ$ to $180^\circ$ were generated and convolved with the normalized image and the maximum response was recorded as $C_{n_i}(x,y)$. The mean value of the normalized image patch at scale $n_i$ before convolution was defined as $I_{n_i}(x,y)$. The output of the line detector at point $(x,y)$ was defined as $C^n(x,y) = C_{n_i}(x,y) - I_{n_i}(x,y)$. The final output image with retinal arterioles and venules enhanced was defined as $O = \sum_{n} C^n + I$, followed by thresholding and denoising to obtain the binarized image (Fig. 1E). An example of corresponding skeletonized image is shown in Figure 1E. "Wrong segmentation" was defined as less than 10% of the first branches from the arcade vessels undiscovred. "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 of vessel within 3-mm ring based on the skeletonized image (Fig. 1D). The equation is as follows:

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

VLD is the vessel length per unit area calculated based on the skeletonized image (Fig. 1D).

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

where $(x, y)$ 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:

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

The vessel density of large vessels was calculated from images (Figs. 1E, 1F) and was termed as PD$_{\text{large}}$ and VLD$_{\text{large}}$. Vessel density of superficial capillaries was labeled as PD$_{\text{cap}}$ and VLD$_{\text{cap}}$.

$$PD_{\text{cap}} = PD - PD_{\text{large}}; \quad VLD_{\text{cap}} = VLD - VLD_{\text{large}}$$

VDI$_{\text{large}}$ and VDI$_{\text{cap}}$ 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, PDcap, VLDcap, VDLcap, PDlarge, VLDlarge, and...

**Figure 1.** Description of the processing of the en-face images acquired from OCTA (3 × 3 mm scan pattern, centered on the fovea). Calculation was performed within the ring between the two yellow circles (the diameter of the outer and inner circle is 3 and 1 mm). (A) Original image. Three circles (100 pixels in diameter) were placed within the FAZ and an averaged intensity was calculated of the circles. (B) Normalized image. The averaged intensity of FAZ was subtracted from the original image. (C) Binarized image of the superficial vasculature. (D) Skeletonized image of the superficial vasculature. (E) Binarized image of segmented retinal large vessels. (F) Skeletonized image of segmented retinal large vessels.
in the model as dependent variables. Only the first acquisition was used for this study.

To compare healthy and diabetic groups, all data were divided into five groups (healthy, wDR, mNPDR, sNPDR, and PDR group) and the comparison of age, SS, PD, PDcap, PDlarge, VLD, VLDcap, VLDlarge, VDI, VDIcap, and VDIlarge among the five groups were conducted using ANOVA. Linear regression model and curve estimation were applied to evaluate the trend of variation for each metric by stage of the disease. Independent variable in this model was the group, which was termed as an ordinal variable according to the severity of the disease stage. In the healthy group, only the first scan was used for analysis. In specific, we also compared the age, SS, and the outcome measurements between healthy and wDR group using independent sample t-test.

We also used the area under the receiver operating characteristic (ROC) curve to evaluate the performance of each metric in detecting mild to moderate diabetic retinopathy.

### Results

#### Demographic Data

In total, 124 OCTA scans of 62 healthy eyes and 129 scans of 68 patients with diabetes were acquired. After screening, 59 eyes from 59 healthy participants (21 eyes in group 1, 24 eyes in group 2, and 14 eyes in group 3) were included as healthy cohort. The mean age of this cohort is 45.3 ± 13.5 (23–68) years, with 36 females. In the diabetic cohort, 118 eyes from 67 patients (mean age 57.1 ± 10.0 [31–81] years, 28 females) were included in the final analysis, with 55 eyes in wDR group, 30 eyes in mNPDR group, 27 eyes in sNPDR group, and 26 eyes in PDR group. All participants were Asians.

#### 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 VLDcap (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, VLD, VLDlarge, VDI, VDIcap, VDIlarge, and VLDcap, respectively. Plots that describe the relationship between disease severity and each metric are displayed in Figure 2.

Compared to healthy group, the VDI, VLD, VDIcap, 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 VDIlarge (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), and 0.955 (95% CI: 0.915–0.996), respectively.

### Table 1. The Mean Values of Measurement of Retinal Superficial Vasculature Within ETDRS Inner Ring and a Comparison Among Different Age Groups

<table>
<thead>
<tr>
<th>Metrics</th>
<th>20 y ≤ Age &lt; 40 y, n = 21</th>
<th>40 y ≤ Age &lt; 60 y, n = 24</th>
<th>Age ≥ 60 y, n = 14</th>
<th>F Value (ANOVA)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SS</td>
<td>9.9 ± 0.3</td>
<td>9.7 ± 0.8</td>
<td>9.5 ± 0.9</td>
<td>1.49</td>
<td>0.235</td>
</tr>
<tr>
<td>PD, %</td>
<td>43.4 ± 1.2</td>
<td>42.5 ± 1.8</td>
<td>42.0 ± 2.0</td>
<td>3.36</td>
<td>0.042</td>
</tr>
<tr>
<td>VLD, mm⁻¹</td>
<td>24.8 ± 1.3</td>
<td>24.2 ± 1.5</td>
<td>23.5 ± 1.7</td>
<td>3.64</td>
<td>0.033</td>
</tr>
<tr>
<td>VDI, μm</td>
<td>17.3 ± 0.4</td>
<td>17.4 ± 0.4</td>
<td>17.7 ± 0.5</td>
<td>3.66</td>
<td>0.032</td>
</tr>
<tr>
<td>PDlarge, %</td>
<td>7.6 ± 1.2</td>
<td>7.8 ± 1.0</td>
<td>7.1 ± 0.9</td>
<td>1.86</td>
<td>0.165</td>
</tr>
<tr>
<td>VLDlarge, mm⁻¹</td>
<td>3.2 ± 0.4</td>
<td>3.0 ± 0.4</td>
<td>3.0 ± 0.4</td>
<td>1.31</td>
<td>0.279</td>
</tr>
<tr>
<td>VDIlarge, μm</td>
<td>23.5 ± 1.1</td>
<td>23.8 ± 1.1</td>
<td>23.2 ± 0.7</td>
<td>1.39</td>
<td>0.257</td>
</tr>
<tr>
<td>PDcap, %</td>
<td>35.8 ± 1.2</td>
<td>34.7 ± 1.7</td>
<td>34.9 ± 2.7</td>
<td>2.18</td>
<td>0.122</td>
</tr>
<tr>
<td>VLDcap, mm⁻¹</td>
<td>21.7 ± 1.2</td>
<td>20.9 ± 1.4</td>
<td>20.4 ± 1.9</td>
<td>3.14</td>
<td>0.051</td>
</tr>
<tr>
<td>VDIcap, μm</td>
<td>16.4 ± 0.5</td>
<td>16.4 ± 0.5</td>
<td>16.9 ± 0.4</td>
<td>6.23</td>
<td>0.004</td>
</tr>
</tbody>
</table>
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

... continuing...
Retinal capillaries become more dilated with aging, while retinal large vessels do not change significantly, with only gender affecting large vessels measurements.

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