Optical coherence tomography angiography analysis of foveal microvascular changes and inner retinal layer thinning in patients with diabetes

Kiyoun Kim, Eung Suk Kim, Seung-Young Yu

ABSTRACT

Aim To evaluate the correlation between inner retinal layer thinning and the foveal microvasculature in type 2 diabetes using optical coherence tomography angiography (OCTA).

Methods A cross-sectional study involved 155 diabetic eyes. All patients were divided into two groups based on diabetic retinopathy (DR) grade: no DR (NDR, n=80) and mild-to-moderate non-proliferative DR (NPDR, n=75). Foveal avascular zone (FAZ) area, FAZ circularity index, FAZ perimeter, vessel density and perfusion index of parafoveal and perifoveal area were calculated using OCTA. The thickness of the macular ganglion cell/inner plexiform layer (mGCIPL) was measured using OCT.

Results In both superficial and deep retinal capillary layers (SRL and DRL), FAZ areas in the NDR (0.38 mm², 0.49 mm²) and NPDR (0.38 mm², 0.48 mm²) were greater than those in the control (0.33 mm², 0.43 mm²). The FAZ circularity index, vessel density and perfusion index in the NDR (0.63, 17.8/mm, 0.32) and NPDR (0.63, 17.5/mm, 0.32) were smaller than those in the control (0.69, 19.6/mm, 0.39). mGCIPL thickness was significantly correlated with FAZ area in the SRL and DRL, as well as with FAZ circularity index, vessel density and perfusion index in the NDR and NPDR. In multivariate regression analysis, the FAZ circularity index (OR=12.2) and vessel density of the parafovea (OR=1.95) were correlated with mGCIPL thinning.

Conclusion OCTA revealed that early foveal microcirculatory alterations in diabetic eyes were related to mGCIPL thickness, regardless of the presence of DR. The decrease in FAZ circularity and parafoveal vessel density were highly correlated with mGCIPL thinning.

INTRODUCTION

Diabetic retinopathy (DR) is the leading cause of blindness in the working-age population worldwide. Early detection and accurate staging of DR are particularly important to prevent sight-threatening complications. In addition, retinal blood flow decreases in patients with type 2 diabetes mellitus (T2DM) who have early-stage DR, suggesting that the retinal microvasculature becomes impaired even before any visible signs of DR occur. Such macular ischaemia induces inner retinal changes, with loss of ganglion cells, attenuation of the inner nuclear layer, and hyalination of the retinal capillary network.

Although fluorescein angiography (FA) remains the gold standard for detecting vascular pathology in the retina, it is invasive and requires exposure to an exogenous contrast agent. Moreover, the axial and lateral resolution of FA only allows for limited visualisation of the capillaries, especially in the macula, where there are overlapping capillary networks. In contrast, optical coherence tomography angiography—OCTA—a new imaging modality that can be combined with en face OCT-based techniques—allows visualisation of the retinal microvasculature without the use of exogenous dyes. OCTA can be used as a non-invasive method for quantifying the dimensions of the retinal capillary networks and foveal avascular zone (FAZ)—a capillary-free zone within the macula that is surrounded by the interconnected capillary beds of the superficial and deep vascular plexuses. Changes in the FAZ dimensions, particularly FAZ enlargement, can indicate the microcirculatory state of the central fovea; such changes are most likely related to macular ischaemia, especially in DR.

Retinal diabetic neurodegeneration (RDN) is regarded as a progressive and degenerative process occurring in the neuroretinal layer that is thought to precede clinically detectable diabetic vasculopathy. Histologically, this manifests as neural apoptosis and glial cell activation—predominately in the ganglion cell layer. These in turn lead to thinning of the macular ganglion cell/inner plexiform layer (mGCIPL), which can be seen by OCT. The purpose of the present study was to analyse alterations in the foveal microcirculation using OCTA, and their correlations with mGCIPL thinning in eyes with no or minimal DR.

METHODS

Study design and population

This study reviewed the medical records of 155 eyes of 155 patients with T2DM who were treated at Kyung Hee University Hospital, Seoul, South Korea. A healthy control group consisted of 75 age-matched patients without DM.

The inclusion criteria were as follows: (1) known diagnosis of T2DM, (2) age between 55 and 65 years, (3) no DR (NDR) or mild-to-moderate non-proliferative DR (NPDR) and (4) OCTA and blood test were performed between May 2016 and April 2017. The exclusion criteria were as follows: (1) clinically significant diabetic macular oedema, (2) previous diagnosis of glaucoma including normal tension glaucoma, (3) ocular hypertension, (4) uveitis, (5) other retinal diseases, (6) any history of retinal treatment (laser photocoagulation, intravitreal injection or vitrectomy) and (7) severe NPDR or PDR (proliferative DR).
The included eye from each participant was selected randomly, unless the eye did not meet the eligibility criteria. The patients with diabetes were divided into two groups on the basis of their DR grade, as determined using fundus photography: the NDR group and the mild-to-moderate NPDR group. NDR was defined as the absence of all features of DR, and mild-to-moderate NPDR was defined in the presence of a microaneurysm, retinal dot haemorrhage or hard exudates, in accordance with the severity scale used in the Early Treatment Diabetic Retinopathy Study.\textsuperscript{10}

Optical coherence tomography
The mGCIPL thickness was measured using the Cirrus HD-OCT 5000 (Carl Zeiss Meditec, Dublin, California, USA); macular scans were carried out using a previously described method.\textsuperscript{9} The mGCIPL map of the Cirrus HD-OCT 5000 occupies the annular area; the inner oval has a horizontal diameter of 1.2 mm and a vertical diameter of 1.0 mm, while the outer oval has a horizontal diameter of 4.8 mm and vertical diameter of 4.0 mm.

Optical coherence tomography angiography
The OCTA images were obtained using the Cirrus HD-OCT 5000 along with Angioplex software. Angioplex operates at 68 000 A-scans per second (wavelength: 840 nm, bandwidth: 90 nm) to acquire one volume scan. All OCTA measurements—both the 3×3 mm and 6×6 mm scans—were performed two times, with two consecutive scan volumes, and the average values were recorded. Acceptable images should have a signal strength greater than 7, minimal motion artefacts and minimal evidence of defocus or blur.

From each of these layers, en face images were generated so that the inner retina was subdivided into two distinct layers: the superficial retinal capillary layer (SRL), located between the inner limiting membrane and the posterior boundary of the IPL, and the deep retinal capillary layer (DRL), which comprises blood vessels between posterior boundary of the IPL and the outer plexiform layer.

In the quantitative analysis (figure 1), the following parameters were evaluated: vessel density and perfusion index (parafovea and perifovea), as well as FAZ area, perimeter and circularity index. Retinal microcirculation parameters were

![Figure 1](http://bjo.bmj.com/first-published-as-10.1136/bjophthalmol-2017-311149 on 19 December 2017, Downloaded from http://bjo.bmj.com/ on March 16, 2022 by guest. Protected by copyright.)
expressed as mean values evaluated within a donut-shaped area using the built-in Cirrus software algorithm (1.5 mm radius from the centre for the parafovea, 3 mm for the perifovea, excluding the central foveal 0.5 mm radius area). The vessel density was defined as the total length of the perfused vessels per unit area within a region of measurement. The perfusion index was the total area of perfused vasculature per unit area within a region of measurement. The built-in analytic algorithm automatically outlined the FAZ boundary along the innermost capillaries, and the area and perimeter of this zone were calculated. FAZ circularity was measured using the following equation: circularity=4πA/P², where A is the area and P is the perimeter. Using this equation, as the shape becomes less round or less smooth, the circularity approaches zero. FAZ area was measured on SRL and DRL, while other OCTA-derived parameters were obtained only from SRL. To measure the FAZ area of the DRL, the generated en face images were imported into Imagej software. The boundary of the FAZ was drawn manually, and the area was calculated. Figure 2 demonstrates an analysis of OCTA parameters and mGCIPL thickness from two NDR patients with and without mGCIPL thinning.

**RESULTS**

A total of 155 eyes, 80 eyes had no clinically observable NDR and 75 eyes had mild-to-moderate NPDR. Baseline demographic data are shown in Table 1. The populations did not differ significantly in terms of mean age. The mGCIPL thickness was significantly lower in the NDR (78.7) and NPDR groups (78.5) than in the control group (82.3); this difference corroborated our previous report. Table 1 also shows the descriptive statistics of the FAZ and microcirculation parameters, measured using OCTA. The mean FAZ area in the SRL (0.38 and 0.38 vs 0.33), DRL (0.58 and 0.59 vs 0.48) and perimeter (2.65 and 2.66 vs 2.40) were larger in the NDR and NPDR groups than in healthy eyes, whereas the mean FAZ circularity index was lower (0.63 and 0.62 vs 0.69). The NDR and NPDR groups also had a significantly lower mean vascular density (parafovea: 17.8 and 17.5 vs 19.6; perifovea: 16.6 and 16.4 vs 18.3) and perfusion index (parafovea: 0.32 and 0.31 vs 0.39; perifovea: 0.34 and 0.34 vs 0.41) than the healthy eyes, in both the parafovea and perifovea. After Bonferroni’s multiple comparisons, post hoc test, mGCIPL thickness and all OCTA-derived parameters are significantly lower in the NDR and NPDR eyes than in the controls. However, there was no difference between the NDR and NPDR eyes.

Table 1 shows that there was significant difference in terms of diabetic duration between NDR (8.3) and NPDR (13.9) groups. Univariate correlation analysis resulted that diabetic duration was significantly correlated with mGCIPL thickness (r=-0.361, P<0.001), FAZ area (r=0.333, P=0.005), FAZ circularity (r=-0.268, P=0.001) and vessel density (r=-0.219, P=0.006). However, mGCIPL thickness and foveal OCTA parameters including FAZ were not different between NDR and NPDR in table 1.

Table 2 shows the Pearson correlations between mGCIPL thickness and FAZ area, FAZ perimeter, FAZ circularity index, vessel density and perfusion index. In both the NDR and NPDR groups, the mGCIPL thickness was strongly correlated with FAZ area in the SRL (coefficients=-0.519, -0.487), FAZ area in the DRL...
Clinical science

The lower quartile, median and upper quartile for mGCIPL thickness were 74.2, 79.0 and 83.5 µm, respectively. When controlled for age, sex, hypertension, blood urea nitrogen, cholesterol, triglyceride, visual acuity, glycated haemoglobin and DR grade, mGCIPL thinning (lower quartile) was significantly associated with the vessel density of the parafovea (OR 0.512, 95% CI 0.455–0.576, P < 0.001).

Table 1 Comparisons of clinical characteristics and foveal microcirculation parameters according to diabetic retinopathy grade in patients with type 2 diabetes

<table>
<thead>
<tr>
<th>Parameter</th>
<th>NDR</th>
<th>NPDR</th>
<th>Control</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No of subjects</td>
<td>80</td>
<td>75</td>
<td>75</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>61.2±4.0</td>
<td>61.8±5.7</td>
<td>61.2±3.9</td>
<td>0.654</td>
</tr>
<tr>
<td>Gender (male/female)</td>
<td>39.41</td>
<td>37.38</td>
<td>36.39</td>
<td>0.995</td>
</tr>
<tr>
<td>Refractive error (dioptres)</td>
<td>−0.39±1.83</td>
<td>−0.37±2.19</td>
<td>−0.47±1.79</td>
<td>0.722</td>
</tr>
<tr>
<td>Presence of hypertension (n)</td>
<td>55</td>
<td>52</td>
<td>17</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Presence of stroke (n)</td>
<td>6</td>
<td>5</td>
<td>2</td>
<td>0.239</td>
</tr>
<tr>
<td>History of insulin treatment (n)</td>
<td>10</td>
<td>25</td>
<td></td>
<td>0.002*</td>
</tr>
<tr>
<td>Diabetic duration (years)</td>
<td>8.3±7.1</td>
<td>13.9±8.5</td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>7.3±1.3</td>
<td>7.8±1.9</td>
<td></td>
<td>0.040*</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>176±44</td>
<td>162±38</td>
<td>187±41</td>
<td>0.002†</td>
</tr>
<tr>
<td>Triglyceride (mg/dL)</td>
<td>178±102</td>
<td>155±89</td>
<td>107±53</td>
<td>0.001†</td>
</tr>
<tr>
<td>BUN (mg/dL)</td>
<td>15.8±4.8</td>
<td>18.4±9.3</td>
<td>15.5±4.0</td>
<td>0.015†</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.74±0.33</td>
<td>0.91±0.76</td>
<td>0.74±0.15</td>
<td>0.067</td>
</tr>
<tr>
<td>Retinal thickness of parafoveal 1–3 mm (µm)</td>
<td>310±18.2</td>
<td>313±19.1</td>
<td>318±16.9</td>
<td>0.051</td>
</tr>
<tr>
<td>mGCIPL thickness (µm)</td>
<td>78.7±6.4</td>
<td>78.5±6.6</td>
<td>82.3±4.9</td>
<td>&lt;0.001†</td>
</tr>
<tr>
<td>LogMAR BCVA</td>
<td>0.04±0.08</td>
<td>0.06±0.09</td>
<td>0.01±0.04</td>
<td>0.007†</td>
</tr>
<tr>
<td>FAZ area (mm²)</td>
<td>0.38±0.11</td>
<td>0.38±0.09</td>
<td>0.33±0.11</td>
<td>0.001†</td>
</tr>
<tr>
<td>Vessel density in SRL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parfovea (3×3 mm)</td>
<td>17.8±1.80</td>
<td>17.5±1.66</td>
<td>19.6±1.39</td>
<td>&lt;0.001†</td>
</tr>
<tr>
<td>Perifovea (6×6 mm)</td>
<td>16.6±1.55</td>
<td>16.4±1.62</td>
<td>18.3±1.22</td>
<td>&lt;0.001†</td>
</tr>
<tr>
<td>Perfusion index in SRL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parfovea (3×3 mm)</td>
<td>0.32±0.03</td>
<td>0.31±0.03</td>
<td>0.39±0.05</td>
<td>&lt;0.001†</td>
</tr>
<tr>
<td>Perifovea (6×6 mm)</td>
<td>0.34±0.05</td>
<td>0.34±0.04</td>
<td>0.41±0.05</td>
<td>&lt;0.001†</td>
</tr>
</tbody>
</table>

*χ² analysis = statistically significant at 5% level of significance.
†One-way analysis of variance.

BCVA, best-corrected visual acuity; BUN, blood urea nitrogen; DRL, deep retinal capillary layer; FAZ, foveal avascular zone; HbA1c, glycated haemoglobin; logMAR, logarithm of the minimum angle of resolution; mGCIPL, macular ganglion cell/inner plexiform layer; NDR, no diabetic retinopathy; NPDR, non-proliferative diabetic retinopathy; SRL, superficial retinal capillary layer.

Table 2 Correlations of foveal avascular zone and foveal microcirculation parameters with macular ganglion cell/inner plexiform thickness in patients with type 2 diabetes

<table>
<thead>
<tr>
<th>mGCIPL thickness</th>
<th>FAZ area</th>
<th>SRL</th>
<th>DRL</th>
<th>FAZ perimeter</th>
<th>FAZ circularity index</th>
<th>Vessel density</th>
<th>Perfusion index</th>
</tr>
</thead>
<tbody>
<tr>
<td>NDR</td>
<td>−0.519*</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>−0.553*</td>
<td>0.517</td>
<td>0.615</td>
<td>0.712</td>
</tr>
<tr>
<td>NPDR</td>
<td>−0.487*</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>−0.429*</td>
<td>0.607</td>
<td>0.480</td>
<td>0.430</td>
</tr>
<tr>
<td>Control</td>
<td>−0.041</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.037</td>
<td>−0.079</td>
<td>0.241</td>
<td>0.251</td>
</tr>
</tbody>
</table>

*Pearson correlation = statistically significant at a 5% significance level.

DRL, deep retinal capillary layer; FAZ, foveal avascular zone; mGCIPL, macular ganglion cell/inner plexiform layer; NDR, no diabetic retinopathy; NPDR, non-proliferative diabetic retinopathy; SRL, superficial retinal capillary layer.


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In this study, we performed a cross-sectional analysis of OCTA-derived parameters, as well as their associations with mGCIPL, in patients with T2DM who had either NDR or mild-to-moderate NPDR. We found that the microcirculatory changes observed with OCTA were prominently correlated with mGCIPL thickness. Moreover, loss of parafoveal vessels and FAZ circularity were highly associated with mGCIPL thinning.

The FAZ is responsible for epicritic vision and central vision. The role of FAZ in macular blood supply status has been widely discussed. Specifically, it is widely accepted that the FAZ size reflects the health of the microcirculation in the retina, and that FAZ measurement could serve an important role in the diagnosis and management of various retinal vascular diseases, such as DR or retinal vein occlusion. OCTA delivers high-resolution, depth-resolved en face images of the macular microvascular network by calculating motion contrast in OCT B-scans acquired repeatedly at the same location. Several recent OCTA analyses have identified alterations in the retinal microvasculature of patients with T2DM, such as distortion and enlargement of the FAZ, retinal capillary dropouts and reduced vessel density.

In both the SRL and DRL of diabetic eyes in the present study, the enlarged FAZ area and loss of FAZ circularity was verified compared with normal eyes. Furthermore, diabetic eyes showed a lower vessel density and perfusion index in the parafovea and perifovea. These findings were consistent with previous OCTA studies involving DR. The FAZ area is enlarged in both DR and retinal vein occlusion due to destruction of the vascular arcades. Prior studies have qualitatively demonstrated that the FAZ becomes more circular in DR, with effects on the FAZ perimeter and circularity index. Thus, loss of FAZ circularity is a good indicator of vascular dropout, and is associated with disease progression in vascular maculopathy. These defects at the FAZ margin are largely due to capillary dropout, as well as macular vascular remodelling that can lead to a decreased vascular density and perfusion index.

In diabetic eyes, the pathophysiology of retinopathy and neuropathy share many of the same triggering mechanisms. Chronic hyperglycaemia-induced chronic low grade inflammation, oxidative stress and ischaemia cause neuronal injury through the elevation of glutamate levels, Muller cell activation and overexpression in the renin-angiotensin system by glial cells. We can assume that thinning of the mGCIPL is developed as a feature of systemic diabetic neuropathy in retina. However, it is not yet clear whether inner retinal thinning induces retinal ischaemia or results from it.

We found that although NPDR group presented significantly longer duration of diabetes than NDR, foveal microcirculation parameters including FAZ were not different between them. This result can be explained that inner retinal thinning and microvascular impairment are expected to be linearly progressed with diabetic duration, however, after dividing into NDR and NPDR groups, previous correlations might become distorted due to confounding factors such as mGCIPL thickness and DR grade. We could assume that when diabetes is first diagnosed, occurrence of inner retinal thinning and microvascular change are not simply dependent on the duration of diabetes.

Our results showed that the OCTA-derived FAZ parameters and microvascular deterioration were associated with mGCIPL thickness (table 2). A similar correlation between FAZ area and mGCIPL thickness has been reported in glaucoma eyes, suggesting that abnormal macular microcirculation occurs at early stage of visual detects. Moreover, FAZ area inversely correlates with central foveal thickness, suggesting that thickened inner retinas requires the FAZ to be smaller to satisfy their substantial metabolic demand. After controlling for confounding factors, we found that parafoveal vessel density and FAZ circularity index were independently related to mGCIPL thinning in the present study (table 3). Similarly, previous studies have suggested that changes in the FAZ circularity and parafoveal vessel density are more reliably correlated with microcirculation impairment in DR. FAZ size shows high variability in healthy eyes, and it is affected by individual differences in ocular magnification such as variation in axial length. To our knowledge, this is the first study to report the relationship between changes of OCTA-derived microvascular parameters and mGCIPL thickness in patients with T2DM. Further studies are required to confirm a longitudinal relationship between neuroretinal degeneration and microvasculopathy in the early stages of DR.

Historically, conventional FA has been the gold standard for evaluating the degree of diabetic macular ischaemia in patients with DR. However, although FA does provide details of the early microvascular changes in the retina (enlargement of the FAZ and intercapillary areas, and decreased capillary perfusion density), screening or frequent assessment using FA is not optimal for the initial stages of DR due to potential adverse effects, as well as the time and cost involved in the procedure. Therefore, OCTA may improve clinical efficiency and is safely tolerated by patients. Measurements of FAZ area and circularity, macular flow index and vessel density using OCTA have high repeatability and reproducibility. In addition, one study showed moderate agreement between OCTA and conventional FA in terms of diabetic macular ischaemia grading.

Another reported reasonable agreement between OCTA and FA. We acknowledge that this study had some limitations regarding its retrospective design. There are varying definitions of the first manifestation of microvascular damage in DR, that is, loss of pericytes on immunochemistry, microaneurysm, focal capillary non-perfusion or loss as clinical sign. Also RDN is observed functionally, as deficits in the electroretinogram, dark adaptation, contrast sensitivity, colour vision and microperimetry. Since we only investigated the correlations of FAZ and capillary density with mGCIPL thickness, our results could not provide conclusive relationship between retinal microvascular change and RDN. Second, we only analysed the foveal microvascular parameters in the SRL. Although both the SRL and DRL are affected by ischaemic change in DR, projection artefacts can result in false measurements of vessel density and the FAZ borders in the DRL using current OCTA technology.

In conclusion, diabetic eyes exhibited significant FAZ enlargement, FAZ distortion and impairment of retinal microcirculation, regardless of the presence of DR. These OCTA-derived

<table>
<thead>
<tr>
<th>Table 3 Multivariate logistic regression analysis of associated factors for the thinning of macular ganglion cell/inner plexiform layer (lower quartile)</th>
<th>OR</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vessel density in SRL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parafovea (3x3 mm)</td>
<td>0.512</td>
<td>0.359 to 0.731</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>FAZ Circularity index</td>
<td>0.822</td>
<td>0.678 to 0.972</td>
<td>0.002</td>
</tr>
</tbody>
</table>

Adjusted for age, sex, duration, hypertension, blood urea nitrogen, cholesterol, triglyceride, visual acuity, glycated haemoglobin and diabetic retinopathy grade. FAZ, foveal avascular zone; SRL, superficial retinal capillary layer.
parameters were correlated with mGCIPL thickness. In particular, FAZ circularity and parafoveal vessel density were promising non-invasive OCTA parameters that could be used to assess the progression of inner retinal layer thinning and future aggressive retinopathy.

Contributors Study concept and design: all authors. Acquisition, analysis and interpretation of data: KK and ESK. Drafting of manuscript: KK. Critical revision of manuscript: KK and S-YY.

Competing interests None declared.

Patient consent Obtained.

Ethics approval The study was approved by the Institutional Review Board of Kyung Hee University Hospital and conformed to the Declaration of Helsinki.

Provenance and peer review Not commissioned; externally peer reviewed.

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REFERENCES


