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ORIGINAL ARTICLE

Progressive retinal neurodegeneration and microvascular change in diabetic retinopathy: longitudinal study using OCT angiography

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Abstract

Aims To investigate the association between progressive macular ganglion cell/inner plexiform layer (mGCIPL) thinning and change of optical coherence tomography angiography (OCTA)-derived microvascular parameters in early-stage diabetic retinopathy (DR).

Methods A retrospective cohort study involved 40 eyes presenting with no DR or mild non-proliferative DR at baseline, and 30 healthy controls were included. All participants underwent spectral-domain OCT and OCTA at baseline and at 6, 12, 18, and 24 months. Change of mGCIPL thickness and OCTA metrics including foveal avascular zone (FAZ) area and FAZ circularity, vessel density (VD), and perfusion index (PI) was measured. Correlations between mGCIPL thickness and OCTA metrics were explored using regression models.

Results Average progressive mGCIPL loss was 0.45 µm per year. Three microvascular parameters were significantly impaired at 24 months compared to baseline (FAZ area: 0.34–0.36 mm^2, VD: 18.9–18.5/mm, PI: 0.35–0.34). A strong positive correlation was found between loss of mGCIPL and VD from baseline to 24 months ($r = 0.817$, $p < 0.001$). Multivariable regression analysis showed that thinner baseline mGCIPL and greater loss of mGCIPL thickness ($B = 0.658$, $p < 0.001$) were significantly associated with change of VD.

Conclusions In the early stage of DR, progressive structural retinal neurodegeneration and parafoveal microvascular change seem to be highly linked. Advanced mGCIPL thinning might precede microvascular impairment in early DR.

Keywords Diabetic retinal neurodegeneration · Ganglion cell/inner plexiform layer · Optical coherence tomography angiography

Introduction

Diabetic retinopathy (DR) is one of the microvascular complications of diabetes mellitus (DM). Development of diabetic macular edema (DME) and proliferative DR (PDR) are major causes of visual impairment [1]. Traditionally, the severity grading of DR has been based on structural changes within inner retinal microvasculature [2], and DR is considered to be a pathology of retinal vascular complications caused by chronic hyperglycemia [3]. However, retinal neurodegeneration has also been emphasized as a crucial and early component of the retinopathy. Diabetic retinal neurodegeneration (DRN) is described as a consequence of neural apoptosis, reactive gliosis, glutamate excitotoxicity, and impairment of neurovascular coupling mechanism [4–6]. Studies with diabetic animal models have shown that increased proinflammatory molecules within the retina triggering the activation of microglial cells and retinal ganglion cell apoptosis precede the development of initial vascular abnormalities such as microaneurysms, vascular loops, and venous beading [7, 8]. Also, in human studies, electroretinography (ERG) and optical coherence tomography (OCT) are able to detect functional and structural DRN features such as inner retinal thinning and delay of implicit time [9–11]. Previous imaging studies in DR patients have

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revealed that retinal blood flow may be decreased and erratic, suggesting dysregulated retinal neurovascular coupling mechanism as a pathogenesis of the initial phases of DR [12, 13].

Fluorescein angiography (FAG), as conventional imaging tools, is not able to clearly visualize small capillary vessels within various retinal layers. Also, FAG is relatively invasive and costly of a procedure to be repeatedly performed on diabetic patients particularly who do not have clinically apparent DR. A recent technology, optical coherence tomography angiography (OCTA), offers quantitative and qualitative retinal blood density and flow without using any contrasts. OCTA can visualize different segmentation slabs such as superficial capillary plexus (SCP) that includes capillary network located in the ganglion cell layer or the nerve fiber layer) and the deep capillary plexus (DCP) slab that consists of the capillary network in the inner nuclear layer (INL) [14]. Studies using OCTA in diabetic patients suggest enlargement and disintegrity of the vascular arcades of the foveal avascular zone (FAZ) and show reduced capillary density [15, 16]. We previously reported that OCTA metrics were significantly correlated with macular ganglion cell/inner plexiform layer (mGCIPL) thinning in patients with early-stage DR [17]. However, temporal relationship between these two components could not be established from a cross-sectional study design.

To the best of our knowledge, our report is the first to evaluate longitudinal microvascular changes in early DR using OCTA. We also analyzed the relationship with decreased mGCIPL thickness to examine whether retinal neurodegeneration may progress with impairments of retinal microvasculature; this may serve as an early ophthalmologic biomarker for neurovascular dysfunction.

Methods

Study design and population

This study was a retrospective cohort study on patients with type 2 DM from Kyung Hee University Hospital, Seoul, South Korea, seen between May 2016 and September 2016. A healthy control group consisted of 30 age-matched patients without DM. The study was approved by the Institutional Review Board of Kyung Hee University Hospital and conformed to the Declaration of Helsinki.

Eligible subjects had either no sign of DR (NDR) or mild non-proliferative diabetic retinopathy (NPDR; ETDRS level 10/10, 10/20, or 20/20) and had completed follow-ups for at least 24 months with consecutive ophthalmic examinations every 6 months. The exclusion criteria were as follows: (1) clinically significant diabetic macular edema; (2) previous diagnosis of glaucoma including normal tension glaucoma (NTG); (3) ocular hypertension; (4) uveitis; (5) other retinal diseases; (6) any history of retinal treatment (laser photocoagulation, intravitreal injection, or vitrectomy). The included eye from each participant was selected randomly unless the selected eye did not meet the eligibility criteria.

Analysis of optical coherence tomography

The mGCIPL thickness was measured using the Cirrus HD-OCT 5000® (Carl Zeiss Meditech, Dublin, CA, USA). The mGCIPL map of the Cirrus HD-OCT 5000 occupied the annular area; the inner circle has a horizontal diameter of 1.2 mm and a vertical diameter of 1.0 mm, while the outer circle has a horizontal diameter of 4.8 mm and vertical diameter of 4.0 mm.

Analysis of optical coherence tomography angiography

The OCTA images were obtained using the Cirrus HD-OCT 5000 along with Angioplex® software (Carl Zeiss Meditech, Dublin, CA, USA). All OCTA measurements (3×3 mm scans) were performed twice, with two consecutive scan volumes, and the average values were recorded. Poor-quality scans with a signal strength index < 8 and significant artifacts were excluded from analysis.

En face images of the SCP were generated from between the inner limiting membrane (ILM) and the posterior boundary of the inner plexiform layer (IPL). In the quantitative analysis, the following parameters were evaluated: vessel density, perfusion index, and FAZ area. Retinal microcirculation parameters were represented as mean values evaluated within a donut-shaped area using the built-in Cirrus software algorithm (1 mm and 3 mm diameter of inner and outer circle from the center for fovea). The vessel density was defined as the total length of the perfused vessels per unit area from a skeletonized vasculature image. The perfusion index was the total area of perfused vasculature per unit area with binarized vasculature image. The built-in analytic algorithm automatically extracted total FAZ area and FAZ circularity index. Figure 1 represents example images of a diabetic subject whose parafoveal vessel density and mean mGCIPL thickness have decreased compared with healthy controls.

Statistical analysis

Statistical analysis was performed using SPSS 18.0 software (SPSS Inc, Chicago, IL). Baseline characteristics were compared with controls using Student's *t* tests and Chi-square tests. Pearson correlations were used to investigate the correlation between the change of OCTA parameters and loss of mGCIPL thickness at 6, 18, and 24 months. The results

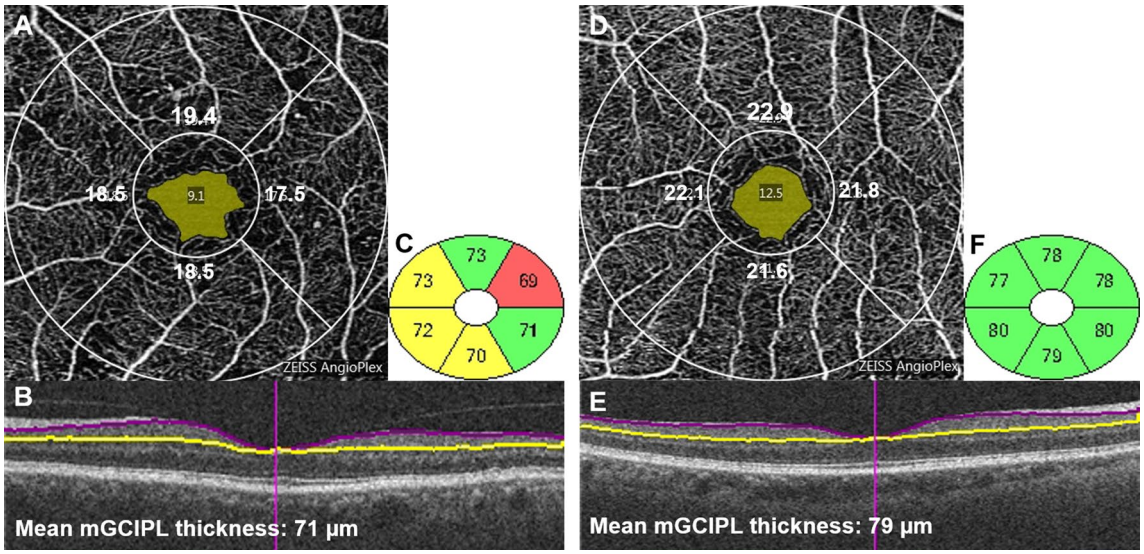

Fig. 1 An example of quantitative analysis in optical coherence tomography angiography (OCTA) and macular ganglion cell/inner plexiform layer (mGCIPL) thickness in a diabetic patient and healthy controls. **a** 3×3 mm macular scan showing the reference plane of the superficial retinal layer (SRL). The area of the foveal avascular zone (FAZ) was automatically calculated (0.29 mm^2) and circularity index was 0.58. The surrounding white circle, which has an outer diameter of 3 mm and an inner diameter of 1 mm, constitutes the parafoveal region. Vessel density was automatically averaged within each quadrant sector (18.5/mm). **b** Boundary lines drawn to measure GCIPL thickness. The purple line indicates the boundary between the retinal nerve fiber layer (RNFL) and ganglion cell layer (GCL) and the yellow line indicates the boundary between the GCL and Inner plexiform layer (IPL). **c** Average of six sectors of the annulus was 71 µm. **d** In healthy controls, corresponding OCTA image shows that area of FAZ was 0.26 mm^2, circularity index was 0.72, and vessel density was 22.1/mm **e, f** The average mGCIPL thickness was 79 µm

of temporal mGCIPL thickness and OCTA parameters were evaluated with a repeated measures ANOVA followed by Bonferroni post hoc tests. Multivariate regression analysis was also performed to find significantly associated factors with change of vessel density at each follow-up.

Results

A total of 40 eyes with NDR or mild NPDR and 30 healthy controls were recruited in this study. Baseline demographic data are shown in Table 1. The DR group showed significantly lower baseline mGCIPL thickness (80.1 vs. 82.3 µm) and greater mGCIPL thinning rate (0.45 vs. 0.22 µm/year) compared with controls. In terms of OCTA-derived parameters, FAZ area (0.36 vs. 0.33 mm^2) was larger, whereas FAZ circularity index (0.58 vs. 0.66), parafoveal vascular density (19.6 vs. 20.8/mm) and perfusion index (0.37 vs. 0.39) were lower than in healthy eyes (all $p < 0.001$). After adjusting for the presence of hypertension, as a potential confounding factor, all four OCTA parameters were still significant different between NPDR and control (all $p < 0.001$).

Figure 2 demonstrates the longitudinal changes in mGCIPL thickness and OCTA parameters over 24 months. The mGCIPL thickness, vessel density, perfusion index decrease, and FAZ area increased through follow-ups. A repeated-measure ANOVA revealed that significant differences in mGCIPL thickness and OCTA parameters between DM and control groups were seen starting at 6–18 months ($p < 0.0125$). The rates of vessel density decrease and perfusion index decrease were 0.368/mm/year and 0.005/year, respectively, over 24 months.

The quantitative correlations between loss of GCIPL thickness and change of four OCTA parameters over 24 months are given in Table 2. There was a strong correlation between decrease in mGCIPL thickness and decreases in vessel density ($r = 0.616$–0.871), and a moderate correlation was obtained with decrease in perfusion index ($r = 0.371$–0.555). No significant correlation was found between two FAZ parameters and mGCIPL thickness. Multiple regression analysis was performed with change of vessel density over 24 months as the dependent variable. Table 3 demonstrates that a greater mGCIPL loss and lower baseline GCIPL thickness are significantly associated with decrease in vessel density after adjusted for age, DM duration, hypertension, blood urea nitrogen, cholesterol, triglyceride, visual acuity, HbA1c, and signal strength intensity.

Table 1 Comparisons of clinical characteristics and foveal microcirculation parameters in patients with type 2 diabetes

	NPDR	Control	*p* value
Eyes (*n*)	40	30	
Age (years)	62 ± 5.5	61.3 ± 4.2	0.574
Gender (male/female)	9:11	17:13	0.795
Hypertension (yes/no)	18:22	10:20	0.021
Diabetic duration (years)	9.4 ± 5.9		
HbA1c (%; mmmol/mol)	7.5 ± 1.2; 58.5 ± 10		
Visual acuity (logMAR)	0.02 ± 0.05	0.01 ± 0.04	0.126
Total cholesterol (mg/dL)	159 ± 41	167 ± 36	0.132
Triglyceride (mg/dL)	145 ± 3.7	147 ± 64	0.781
BUN (mg/dl)	16.1 ± 38	16.8 ± 4.6	0.512
Creatinine (mg/dl)	0.74 ± 0.33	0.76 ± 0.21	0.767
mGCIPL thickness (μm)	80.1 ± 6.7	82.3 ± 5	0.008*
Rate of mGCIPL thinning (μm/year)	0.45 ± 0.57	0.22 ± 0.31	< 0.001*
FAZ Area (mm^2)	0.36 ± 0.09	0.33 ± 0.11	< 0.001*
FAZ circularity index	0.58 ± 0.07	0.66 ± 0.07	< 0.001*
Macular vessel density (/mm)	19.6 ± 1.4	20.8 ± 1.1	< 0.001*
Macular perfusion index	0.37 ± 0.02	0.39 ± 0.02	< 0.001*

Data presented as *n* or mean ± standard deviation unless otherwise indicated

BUN blood urea nitrogen, *FAZ* foveal avascular zone, *HbA1c* glycosylated hemoglobin, *mGCIPL* macular ganglion cell/Inner plexiform layer, *NPDR* non-proliferative diabetic retinopathy

*Statistically significant when $p < 0.0125$, according to Student *t* test

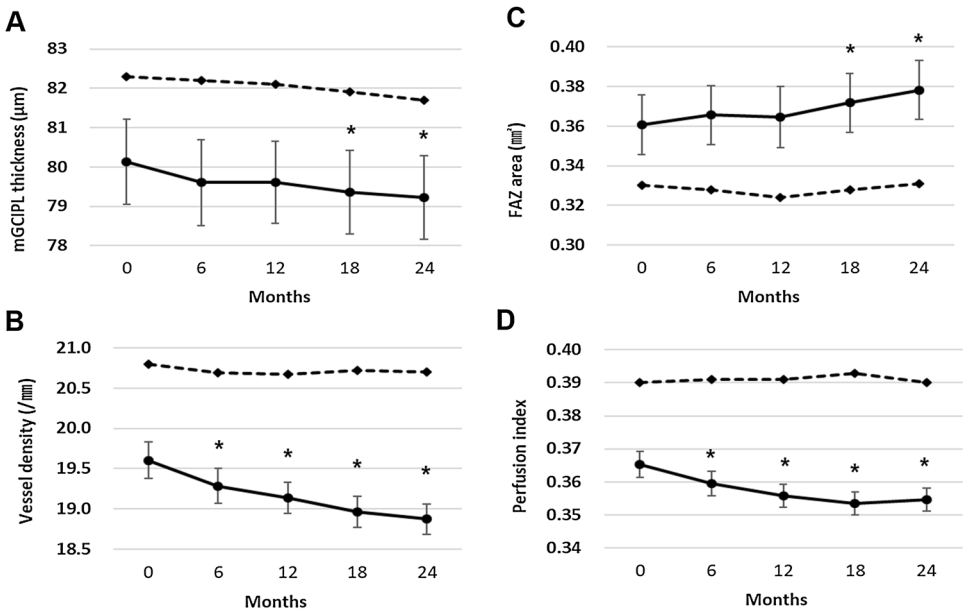

Fig. 2 A longitudinal change of macular ganglion cell/inner plexiform layer (mGCIPL) thickness and optical coherence tomography angiography (OCTA) parameters over 24 months. **a** mGCIPL thickness, **b** vessel density, **c** perfusion index decrease, and **d** foveal avascular zone area increases through follow-up. A significant difference compared to baseline was marked with * at each point when was $p < 0.0125$. Dotted line represents values of healthy controls, while the solid line represents those in the diabetic group

Discussion

Neurodegeneration is demonstrated as an early process in DR and known to precede clinically visible retinal vasculopathy. Recent advanced imaging techniques have allowed identification of early changes in diabetic retina which manifest before abnormal fundoscopic findings. OCT studies have demonstrated that the retinal nerve fiber, ganglion cell, and inner plexiform layers are thinner in DR patients, also suggesting death of neurons in the inner retina before that of vascular cells [18, 19]. Implicit time is delayed in diabetic patients with or without NPDR, and amplitude alterations have been observed in ERG studies [20, 21]. At the cellular level of progressive DRN, neuronal stress and cell apoptosis also lead to glial activation.

Table 2 Correlations between loss of macular ganglion cell/inner plexiform thickness and change of optical coherence tomography angiography parameters over 24 months

Loss of mGCIPL thickness	Change of FAZ area	Change of FAZ circularity index	Change of vessel density	Change of perfusion index
6 month				
Coefficient	−0.254	0.08	0.219	0.395[a]
p value	0.124	0.632	0.187	0.014
12 month				
Coefficient	−0.064	−0.013	0.616[a]	0.371[a]
p value	0.701	0.937	<0.001	0.022
18 month				
Coefficient	0.025	0.026	0.723[a]	0.478[a]
p value	0.881	0.875	<0.001	0.002
24 month				
Coefficient	−0.105	−0.05	0.871[a]	0.555[a]
p value	0.532	0.767	<0.001	<0.001

[a]Pearson correlation = statistically significant at a 5% significance level

FAZ foveal avascular zone, *mGCIPL* macular ganglion cell/inner plexiform layer

Activated glia may not properly regulate retinal blood flow or maintain the blood retinal barrier but may secrete pro-inflammatory cytokines which aggravate retinal microvascular dysfunction [22]. In addition, retinal endothelial cells and pericytes are damaged by inflammatory mediators from surrounding neurons and glia cells, which first affects capillary loss or microaneurysm observed in early stage of DR [22–24]. Indeed, several authors have demonstrated that reduced blood flow in the choroid and retina causes chronic ischemia in RPE and neuroretina [25, 26]. This longitudinal study aimed to evaluate the temporal relationship between the reduction in superficial vessel density and a reduction in neuroretinal thickness in order to clarify the causal relationship.

DR is now understood to be a part of systemic sensory neuropathy. Retinal neurodegeneration in diabetic patients is caused by complex mechanisms including both ocular and systemic factors. Bresnick et al. [27] suggested that DR could be regarded as a neurosensory disorder resulting from metabolic and systemic damage to the retina and the clinically observed vascular abnormalities. We previously reported clinical characteristics and systemic risk factors associated with mGCIPL thinning in long-established DM [28]. Specifically, degree of systemic neuropathy was significantly related with mGCIPL thickness in early-stage DR [29].

The utilization of objective tests for early detection of neuroretinal dysfunction allows prediction of sight-threatening diabetic retinopathy. Multifocal ERG (mfERG) implicit time abnormalities are locally predictive of new diabetic retinopathy development and diabetic macular edema [30]. Thinning of the inner neuroretinal layer has been reported as the primary OCT finding in diabetic patients with no DR. In a longitudinal study, inner retinal layer thinning progressed during the 1-year follow-up [31]. The reduction rate was 0.53 mm per year [32] and was correlated with functional retinal alterations [33]. Progressive loss of mGCIPL is an independent risk factor for DR progression in early-stage DR [10], while outer retinal layers were less affected than the inner layers before DR development [34].

There has been increasing evidence that OCTA can detect subclinical changes or, more accurately, quantify early-stage DR, whereas FAG is not recommended or useful [35]. In DR, the FAZ area is enlarged and irregularly shaped as a result of interruption and loss of the foveal vascular network compared with controls [36, 37]. Alterations in the microcirculation may precede clinically distinguishable retinopathy in diabetic patients. Therefore, quantitative assessment of microvascular status in DR screening can detect subclinical DR changes that would be crucial in determining earlier intervention and prevention of future vision loss. Some studies have found that vessel density in the SCP and DCP on OCTA is lower in diabetic patients without DR [38, 39].

Table 3 Multiple regression analysis for descriptive variables associated with the change of parafoveal vessel density over 24 months

Change of vessel density	12 months	18 months	24 months
Loss of mGCIPL thickness			
Odds ratio (95% CI)	0.411 (0.256–0.567)	0.495 (0.361–0.629)	0.589 (0.462–0.716)
p value	<0.001	<0.001	<0.001
Baseline mGCIPL thickness			
Odds ratio (95% CI)	−0.034 (−0.066 to −0.002)	−0.029 (−0.052 to −0.005)	−0.026 (−0.047 to −0.005)
p value	0.036*	0.017*	0.018*

*Adjusted for age, sex, duration, hypertension, BUN, cholesterol, triglyceride, visual acuity, HbA1c, and signal strength intensity

CI confidence interval, *mGCIPL* macular ganglion cell/inner plexiform layer

In this study, diabetic eyes had significantly lower vessel density and larger FAZ area than healthy subjects, consistent with previous literatures. Macular VD may predict the severity of DR with high sensitivity, as it was found to be negatively correlated with DR severity [40]. Even, two FAZ parameters were significantly impaired in early DR, and their correlations with mGCIPL thinning were not significant in current study. This can be explained by considering the anatomy of the normal retinal ganglion cell distribution in macular region, that ganglion cell layer becomes very thin and difficult to detect accurately in fovea.

As we obtained parafoveal vessel density within a donut-shaped area using the built-in algorithm (1 mm and 3 mm diameter of inner and outer circle), effect of FAZ enlargement or remodeling could be excluded from the calculations of vessel density. Instead, retinal ischemia may arise from damage to the retinal capillaries early in the disease process as a result of damage to the endothelial cells and pericyte loss. Fu et al. [41] explained how expanding capillary segment nonperfusion due to progressive hypoxia could lead to the larger areas of nonperfusion that follows in NPDR and PDR. In addition, Alibhai et al. [42] measured nonperfusion area using wide-field OCTA and found decreased capillary perfusion with increasing DR severity.

Moreover, we previously compared the vessel density of SCP and DCP with mGCIPL thickness and found significant positive correlation between thickness and vessel density [17]. There are still very limited data on the direct correlations between retinal layer thickness changes and microvascular changes detected on OCTA, that particularly occurred in preclinical stage of DR. Vujosevic et al. [43] reported that perifoveal capillary loss in the SCP with OCTA was highly correlated with inner retinal thinning in early DR. They also published that early changes in the peripapillary vessel morphology and VD of the retinal peripapillary area in patients with DM without DR that correlate to NFL thinning [44]. However, these cross-sectional design studies are limited to establish the decrease in VD as a surrogate marker for DR progression. It is also unclear whether neural changes in the inner retina precede early retinal microvascular changes or follow retinal microvascular changes or whether they occur in parallel. We found a strong correlation between decrease in mGCIPL thickness and decreases in vessel density ($r = 0.616$–0.871), while only a moderate correlation with decrease in perfusion index ($r = 0.371$–0.555). VD is calculated from skeletonized image which normalizes the diameter of capillary vessels, removing the influence of vessel size on retinal perfusion measurements. Rosen et al. [45] noted that perfusion capillary density rather increases in no-DR group due to autoregulatory response to increased metabolic demand, while the decrease in PCD that follows in NPDR and PDR results largely from loss of capillary segments. Consistent with this hypothesis, the correlations between decrease in perfusion index and mGCIPL loss was also weaker than that of vessel density in our results.

Diagnostic algorithms and management protocols are still based on funduscopic examinations. Current therapies on DR have been limited to advanced stage of DR, such as PDR or DME, when visual impairment and neurodegeneration is already in progress. In the future, the key to preventing all or most of the impact of diabetes on vision will likely be the development of therapeutic agents that prevent early retinopathy or the subclinical pathological vascular and neural changes that underlie the early functional abnormalities. Therefore, further longitudinal studies are warranted to define the predictability of these diagnostic modalities and supplement routine clinical practice when early assessment and intervention are required.

Some limitations to our results exist. The observation period was not sufficient to define a temporal relationship between mGCIPL loss and decrease in vessel density. We also acknowledged that given the pilot study with small number of subjects, it is still necessary to be cautious before generalizing results. As another limitation, DCP slab OCTA image was not included in the analysis because several projection artifacts prevented constant qualified image quality over 24 months. Recent OCTA studies on microvascular changes of early DR have reported different results in terms of SCP and DCP layer. Choi et al. [25] documented retinal microvascular abnormalities in both SCP and DCP were significantly different in patients with DM. Vujosevic et al. [43] suggested that microvascular change of DCP is more precociously occurred in patients with T2DM. In the future, we expect projection artifacts removal algorithms which can minimize these artifacts in the DCP layers would provide details of early microvascular changes in DCP.

In conclusion, we recognized longitudinal change of OCTA metrics in diabetic eyes which resulted in significant impairment from 6 to 18 months on (when compared to controls). Progressive loss of mGCIPL thickness was strongly correlated with a decrease in vessel density in the SCP. Lastly, early microvascular damage was likely to develop in diabetic patients who showed rapid GCIPL loss or low baseline GCIPL thickness. Therefore, our results suggest that early detection of subclinical DR based on loss of mGCIPL thickness could provide timely recognition and management for patients at a greater risk of further microvascular impairment in DR. Given that low mGCIPL thickness is an independent risk factor for decrease in vessel density, mGCIPL thinning seems to occur before microvascular change in early DR. Rather, we wish to offer a more cautious approach, that number of subjects and follow-up duration were not enough to provide statistically significant temporal relationship between two parameters. We would expect that more definitive conclusion would be suggested by further larger prospective studies.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval The study was approved by the Institutional Review Board of Kyung Hee University Hospital.

Informed consent For this type of study, formal consent is not required.

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