The Ganglion Cell-Inner Plexiform Layer Thickness/Vessel Density of Superficial Vascular Plexus Ratio According to the Progression of Diabetic Retinopathy

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Purpose. The purpose of this study was to identify the relationship between the gangion cell-inner plexiform layer (GC-IPL) and retinal vasculature in the context of the progression of diabetic retinopathy (DR).

Methods. The subjects were divided into four groups according to DR stage: normal controls (group 1), patients with diabetes mellitus (DM) without DR (group 2), patients with mild or moderate nonprogressive DR (NPDR; group 3), and patients with severe NPDR (group 4). GC-IPL thickness, vessel density of superficial vascular plexus (SVD), and the GC-IPL/SVD ratio were compared among the groups.

Results. A total of 556 eyes were enrolled; 288 in group 1, 140 in group 2, 76 in group 3, and 52 in group 4. The mean GC-IPL thicknesses were 83.57 ± 7.35, 82.74 ± 7.22, 81.33 ± 6.74, and 79.89 ± 9.16 μm in each group, respectively ($P = 0.006$). The mean SVDs were 20.40 ± 1.26, 19.70 ± 1.56, 18.86 ± 2.04, and 17.82 ± 2.04 mm^{-1} in each group, respectively ($P < 0.001$). The GC-IPL/SVD ratios were 4.11 ± 0.38, 4.22 ± 0.40, 4.36 ± 0.54, and 4.54 ± 0.55 in each group, respectively ($P < 0.001$). In Pearson's correlation analysis, DR stage was significantly correlated with the GC-IPL/SVD ratio (coefficient = 0.301; $P < 0.001$).

Conclusions. As the DR stage progressed, the GC-IPL thickness tended to decrease, with the macular SVD showing a significant reduction. Additionally, the impairment of retinal vasculature was more prominent than GC-IPL thinning as DR progressed, which suggests that retinal vasculature changes may precede diabetic retinal neurodegeneration.

Keywords: ganglion cell-inner plexiform layer, vessel density, diabetic retinopathy

Diabetic retinopathy (DR) is a major complication of diabetes mellitus (DM), with 93 million cases being estimated globally.[1] DR, which is the leading cause of blindness in the working-age population, causes damage to the retina of various forms.[2,3] Diabetic retinal neurodegeneration (DRN) is a representative type of retinal damage caused by DR. DRN leads to abnormalities in the neural retina and capillary bed located in the inner retina by the activation of polyol and hexosamine pathologic pathways, de novo synthesis of diacylglycerol-protein kinase C, and the production of free radicals and advanced glycation end-products.[4,5] Thus, the ganglion cell-inner plexiform layer (GC-IPL) of patients with DR is thinner than that of healthy people, which has been reported in many previous studies.[6–9]

Previous studies reported impaired macular circulation using analyses of the hemodynamics of the macular area in patients with diabetes.[10,11] With the development of optical coherence tomography angiography (OCTA), which enables visualization of the fine retinal microvasculature of multiple layers, several studies have reported impairment of the retinal microvasculature in patients with DM and DR. Cao et al.[12] found that the parafoveal vessel density (VD) of both the superficial and deep capillary plexuses was reduced in patients with DM. Nesper et al.[13] reported that retinal and choriocapillaris vascular nonperfusion in OCTA was correlated significantly with disease severity in eyes with DR. As such, DR can cause both GC-IPL thinning and microvasculature impairment, and these two types of damage are known to be related to each other.[7,14] Inner retinal reduction can cause microvasculature impairment, and impaired retinal perfusion can lead to inner retinal atrophy. Therefore, a better understanding of the relationship between the GC-

IPL and retinal vasculature in patients with DR is expected to provide insight into the retinal damage progression caused by DR.

The purpose of this study was to identify the relationship between the GC-IPL and retinal vasculature in the context of progression of DR via analyses of the GC-IPL thickness, macular VD of superficial vascular plexus (SVD), and GC-IPL/SVD ratio in patients with DR.

Methods

Patients

This retrospective, cross-sectional study was performed according to the tenets of the Declaration of Helsinki and was approved by the Institutional Review Board/Ethics Committee of Chungnam National University Sejong Hospital, Sejong, Republic of Korea. The study enrolled subjects aged ∼ 40–69 years who visited the retina clinic of Chungnam National University Hospital for regular checkups. The requirement for informed consent was waived owing to the retrospective nature of the study. We recorded detailed histories and measurements of best-corrected visual acuity (BCVA), intraocular pressure, spherical equivalent, axial length, fluorescein angiography (HRA Spectralis; Heidelberg Engineering, Inc., Heidelberg, Germany), spectral-domain optical coherence tomography (SD-OCT), and OCTA. DR was graded according to the International Clinical Diabetic Retinopathy Disease Severity scale, which was based on findings observable in dilated ophthalmoscopy.[15] The subjects were divided into four groups according to the DR stage: normal controls (group 1), patients with DM without DR (group 2), patients with mild or moderate nonprogressive DR (NPDR; group 3), and patients with severe NPDR (group 4). The exclusion criteria were a history of retinal disease other than DR, macular edema with central macular thickness >300 μm or cystic changes, progressive DR, glaucoma, history of ocular trauma, any prior intraocular surgery other than cataract extraction, presence of any ocular disease including corneal disease, retinal abnormalities, neuro-ophthalmic disease, intraocular pressure >21 mm Hg, axial length >26.0 mm, and BCVA <20/25. If both eyes met the inclusion criteria, one eye was randomly selected for the study.

GC-IPL Thickness, Retinal Vasculature Parameters, and GC-IPL/SVD Ratio Measurements

The GC-IPL thickness was measured with a 512 × 128 macular cube scan based on an algorithm of the Ganglion Cell Analysis module of the Cirrus HD OCT 5000 instrument (Carl Zeiss Meditec, Dublin, CA, USA). The algorithm automatically measured GC-IPL thickness by identifying the outer boundaries of the retinal nerve fiber layer (RNFL) and IPL of the macula using three-dimensional information from the macular cube. Average and six-sector GC-IPL thicknesses were measured, the images with segmentation errors were excluded from the analysis.

Retinal vasculature parameters were measured using the Cirrus HD-OCT 5000 instrument with AngioPlex software (Carl Zeiss Meditec), at a wavelength of 840 nm and the acquisition of 68,000 A-scans per second. The instrument achieves sensitivity and accuracy by incorporating an optical microangiography (OMAG) algorithm and retinal tracking technology. We obtained a foveal centered scan area of 3 × 3 mm pattern, and en face OCTA images were generated automatically by the OMAG algorithm of AngioPlex software. The VD (total length of perfused vasculature per unit area) and perfusion density (PD; total area of perfused vasculature per unit area) of the superficial vascular plexus, which spanned from the internal limiting membrane to the IPL, were measured automatically by the software and two investigators (authors L.M.W. and L.H.B.) confirmed the segmentation accuracy. The 3 × 3 mm^2 scan was composed of a 1-mm center and 4-quadrant sectors that were identical to the inner circles of the Early Treatment of Diabetic Retinopathy Study (ETDRS). The central area was a 1-mm-diameter central circle, surrounded by a ring showing the average of the 4-quadrant sectors, and the full area was demarcated by a 3-mm-diameter circle, equivalent to the inner circle of the ETDRS. All images were individually reviewed for quality by two investigators (authors L.M.W. and L.H.B.); images with loss of fixation or foveal centration, segmentation errors, motion artifacts, or a signal strength of less than nine (range = 0 to 10) were excluded.

The GC-IPL/SVD ratio was calculated by dividing the mean GC-IPL thickness by the full area of the SVD.

Statistical Analysis

Baseline demographics, and OCT and OCTA measurements were compared using one-way analysis of variance, followed by a post hoc test (Bonferroni test). The chi-squared test was used for comparing categorical data. Pearson's correlation analysis was performed to identify the relationship between DR stage and the GC-IPL/SVD ratio. All statistical analyses were performed using SPSS software (version 22.0; IBM Corp., Armonk, NY, USA).

Results

A total of 556 eyes were enrolled; 288 in group 1, 140 in group 2, 76 in group 3, and 52 in group 4 (Table 1). The mean age of each group was 59.61 ± 7.11, 60.23 ± 7.20, 60.47 ± 5.02, and 62.10 ± 6.38 years, respectively ($P = 0.102$). The HbA1c levels of group 2, group 3, and group 4 were 7.10

Table 1. Demographics and Clinical Characteristics of the Subjects

	Group 1($N = 288$)	Group 2($N = 140$)	Group 3($N = 76$)	Group 4($N = 52$)	*P* Value
Age (mean ± SD, y)	59.61 ± 7.11	60.23 ± 7.20	60.47 ± 5.02	62.10 ± 6.38	0.102
Sex (male, %)	173 (60.1)	80 (57.1)	39 (51.3)	33 (63.5)	0.468
HbA1c level (mean ± SD, %)	n/a	7.10 ± 1.01	7.69 ± 1.08	7.43 ± 1.26	0.056
BCVA (mean ± SD, logMAR)	−0.02 ± 0.07	−0.01 ± 0.05	0.01 ± 0.08	−0.01 ± 0.08	0.096
SE (mean ± SD, diopters)	0.02 ± 1.46	−0.07 ± 1.35	−0.03 ± 1.03	0.09 ± 1.33	0.866
IOP (mean ± SD, mm Hg)	15.88 ± 3.02	15.89 ± 2.50	15.63 ± 2.50	16.19 ± 2.84	0.259
Axial length (mean ± SD, mm)	23.73 ± 0.78	23.58 ± 0.84	23.63 ± 0.67	23.60 ± 0.85	0.283
Cup/disc ratio (mean ± SD)	0.54 ± 0.14	0.55 ± 0.16	0.54 ± 0.14	0.58 ± 0.14	0.386

BCVA, best-corrected visual acuity; SE, spherical equivalent; IOP, intraocular pressure.

Table 2. Comparison of the Ganglion Cell-Inner Plexiform Layer Thickness Among Groups

	Group 1	Group 2	Group 3	Group 4	*P* value
Average	83.57 ± 7.35	82.74 ± 7.22	81.33 ± 6.74	79.89 ± 9.16	**0.006**
Superior	85.11 ± 7.17	83.56 ± 8.65	81.92 ± 6.85	80.53 ± 8.53	**0.005**
Superotemporal	83.93 ± 8.12	82.10 ± 9.27	81.17 ± 8.58	79.65 ± 9.19	**0.010**
Inferotemporal	83.25 ± 6.77	82.86 ± 8.21	82.42 ± 8.21	80.55 ± 8.21	**0.045**
Inferior	81.96 ± 8.43	80.86 ± 9.78	79.98 ± 7.48	77.51 ± 10.32	**0.002**
Inferonasal	84.19 ± 7.84	83.50 ± 7.52	81.51 ± 8.56	80.38 ± 9.19	**0.007**
Superonasal	87.01 ± 7.82	85.64 ± 9.13	83.11 ± 7.81	81.47 ± 8.20	**<0.001**

Values in boldface ($P < 0.050$) are statistically significant.

Table 3. Comparison of the Parameters of Retinal Microvasculature Using Optical Coherence Tomography Angiography Among Groups

	Group 1	Group 2	Group 3	Group 4	*P* Value
Vessel density (mm^{-1})					
Center	10.19 ± 2.89	9.34 ± 3.09	8.93 ± 2.36	8.24 ± 2.40	**<0.001**
Ring	23.75 ± 1.27	21.97 ± 2.15	19.40 ± 4.13	19.25 ± 2.22	**<0.001**
Full	20.40 ± 1.26	19.70 ± 1.56	18.86 ± 2.04	17.82 ± 2.04	**<0.001**
Perfusion density (%)					
Center	17.86 ± 5.16	16.75 ± 5.83	15.96 ± 4.02	15.20 ± 4.62	**<0.001**
Ring	39.14 ± 2.02	37.67 ± 3.98	37.18 ± 3.51	36.37 ± 3.49	**<0.001**
Full	36.70 ± 2.01	35.77 ± 2.36	34.81 ± 3.41	34.05 ± 3.15	**<0.001**

Values in boldface ($P < 0.050$) are statistically significant.

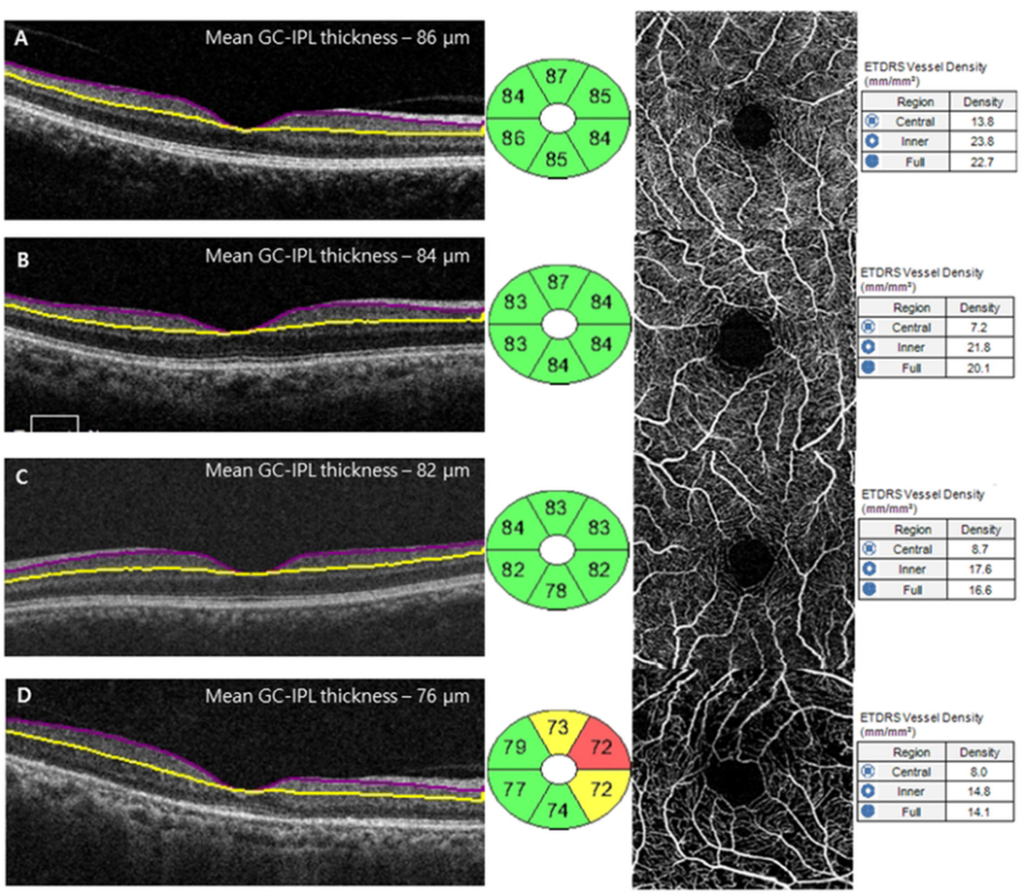

Figure 1. Representative B-scan images with ganglion cell-inner plexiform layer (GC-IPL) thickness and 3 × 3 mm^2 optical coherence tomography angiography images of superficial vascular plexus with vessel density (SVD) in group 1 (**A**), group 2 (**B**), group 3 (**C**), and group 4 (**D**). As the stage of diabetic retinopathy progresses, the GC-IPL thickness and SVD tend to decrease, and the GC-IPL/SVD ratio tends to increase (**A**, GC-IPL/SVD ratio = 3.79; **B**, GC-IPL/SVD ratio = 4.18; **C**, GC-IPL/SVD ratio = 4.94; **D**, GC-IPL/SVD ratio = 5.39).

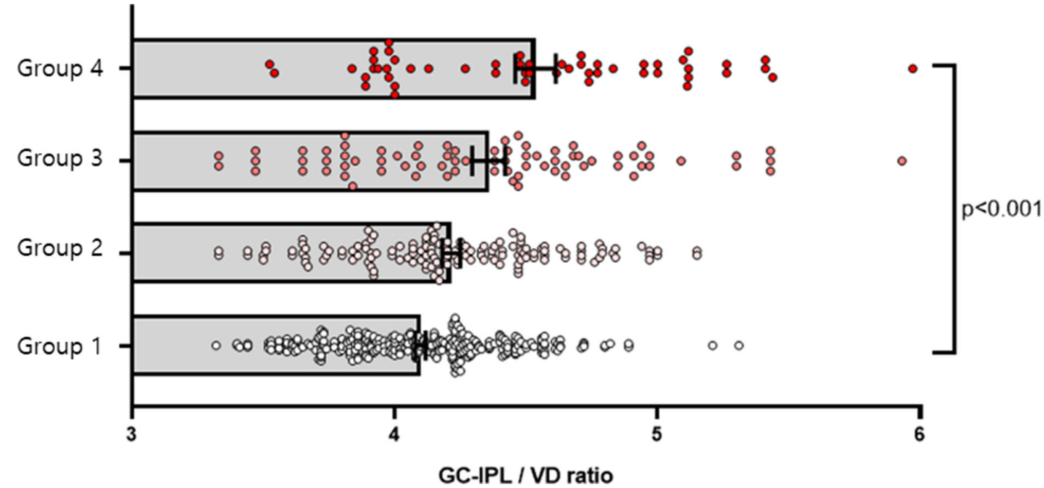

Figure 2. Scatter plots and bar graphs (mean ± standard errors) of the ganglion cell-inner plexiform layer/superficial vascular plexus vessel density ratio of each group: group 1, normal controls; group 2, patients with diabetes without diabetic retinopathy (DR); group 3, patients with mild or moderate nonprogressive DR; group 4, patients with severe nonprogressive DR.

± 1.01, 7.69 ± 1.08, and 7.43 ± 1.26 %, respectively (P = 0.056). Other factors, including sex, BCVA, spherical equivalent, intraocular pressure, axial length, and the cup/disc ratio were also not significantly different among groups.

The mean GC-IPL thicknesses were 83.57 ± 7.35, 82.74 ± 7.22, 81.33 ± 6.74, and 79.89 ± 9.16 μm in group 1, group 2, group 3, and group 4, respectively, which was significantly different ($P = 0.006$; Table 2). In post hoc analyses, group 4 showed a significantly thinner GC-IPL than group 1 ($P = 0.016$). The GC-IPL thickness of the other groups showed a tendency to decrease as the DR grade increased; however, post hoc analyses did not reveal a statistically significant difference. The results for sectoral GC-IPL thickness were similar to those for mean GC-IPL thickness, which were shown in Table 2.

The mean SVDs of the full area were 20.40 ± 1.26, 19.70 ± 1.56, 18.86 ± 2.04, and 17.82 ± 2.04 mm^{-1} in group 1, group 2, group 3, and group 4, respectively, which was significantly different ($P < 0.001$; Table 3). In post hoc analyses, SVD showed a statistically significant decrease as the DR grade increased (group 1 vs. group 2, $P < 0.001$; group 2 vs. group 3, $P = 0.001$; and group 3 vs. group 4, $P = 0.001$; Fig. 1). The mean superficial vascular plexus PDs (SPD) of the full area were 36.70 ± 2.01, 35.77 ± 2.36, 34.81 ± 3.41, and 34.05 ± 3.15% in each group, respectively ($P < 0.001$). In post hoc analyses, SPD also showed a statistically significant decrease as the DR grade increased, similar to SVD, with the exception of group 3 vs. group 4 ($P = 0.504$).

The GC-IPL/SVD ratios were 4.11 ± 0.38, 4.22 ± 0.40, 4.36 ± 0.54, and 4.54 ± 0.55 in group 1, group 2, group 3, and group 4, respectively, which was significantly different ($P < 0.001$; Fig. 2). In post hoc analyses, the group 1 vs. group 3 ($P < 0.001$), group 1 vs. group 4 ($P < 0.001$), and group 2 vs. group 4 ($P < 0.001$) comparisons showed significant differences. In Pearson's correlation analyses, DR stage was significantly correlated with the GC-IPL/SVD ratio (coefficient = 0.301; $P < 0.001$).

Discussion

In this study, we evaluated GC-IPL thickness and retinal superficial vascular plexus using OCTA, according to DR stage. We found that patients with DM had a thinner GC-IPL and lower macular SVD compared with healthy individuals, and as the DR stage progressed, GC-IPL thickness tended to decrease; macular SVD showed a significant decrease. Additionally, the GC-IPL/SVD ratio showed a significant increase as DR worsened, indicating that retinal vascular impairment was more prominent than inner retinal layer structural damage as DR progressed.

Lim et al.[16] reported that the estimated reduction rates of the GC-IPL thickness in the no-DR (-0.627 μm/year) and NPDR (-0.987 μm/year) groups were 2.26-fold (P = 0.010) and 3.56-fold (P = 0.001) faster, respectively, than in the control group. Ng et al.[9] reported GC-IPL loss in patients with DR; the loss was progressive in advanced DR with a decrease in inner retinal layer thickness. Our study also showed that DR patients had a thinner GC-IPL than normal controls, and it also tended to be thinner as the DR stage progressed. A reduction in GC-IPL thickness is associated with DRN, which causes neural apoptosis and reactive gliosis in astrocytes and Muller cells.[4,8,17] DRN can also cause impairment of neurovascular coupling and breakdown of the blood-retinal barrier due to glutamate accumulation, which increases the secretion of vascular endothelial growth factor.[16,18–21] Therefore, besides the impairment in visual function caused by neural tissue damage, DRN can also affect DR progression.[22] Meanwhile, DRN is associated with other neurodegenerative diseases, especially Alzheimer's disease.[23] DRN and Alzheimer's disease share pathogenic pathways, including insulin signaling impairment, low-grade inflammation, accumulation of advanced glycation end-products, and increased oxidative stress.[8,23] As such, GC-IPL thickness could be a useful biomarker not only of DR progression but also the risk of Alzheimer's disease in patients with DR.

Nesper et al.[13] found a strong correlation between DR severity and nonperfusion of the retinal capillary plexuses using OCTA. They reported that retinal VD measured at the superficial capillary plexus, deep capillary plexus, and full retina decreased significantly with DR severity, and the area percentage of nonperfusion measured in the superficial capillary plexus, deep capillary plexus, full retina, and choriocapillaris increased significantly with increasing severity of

DR. Our study showed significant reductions in the SVD as DR progressed, consistent with the previous study. Although the SPD showed a similar trend to the SVD, group 3 and group 4 did not show a significant SPD difference in post hoc analyses, indicating that SVD may be a more sensitive parameter to identify the changes in vascular flow than SPD for patients with the advanced stage of DR. Considering that there was no abnormality associated with DR on fluorescein angiography in both the normal controls and patients with DM without DR, it is noteworthy that there were significant differences in SVD and SPD between the two groups 3 and 4. Bucolo et al.[24] reported that diabetic mice with the retinal protein expression of VEGF-A, HIF-1α, and PKCβ/HuR pathway showed a decreased RNFL thickness at 9 and 46 weeks of age, whereas no difference in the retinal vasculature was observed by fluorescein angiography compared to the control group. Taken together, OCTA may be more sensitive to microvascular impairment than fluorescein angiography in the early stages of DR. Although OCTA may not completely replace fluorescein angiography due to the lack of functional testing of the retinal vasculature, it can replace invasive fluorescein angiography to some extent in investigations and follow-up of microvasculature impairment in DR.

Lee et al.[7] reported that GC-IPL thickness was significantly associated with macular VD in patients with type 2 diabetes. Vujosevic et al.[14] also found significant correlations between OCTA parameters, including VD and PD, and RNFL thickness in patients with DR. As such, the inner retinal layer and retinal microvasculature are connected to each other by neurovascular coupling, and each type of damage may affect each other.[4,8] In our study, the GC-IPL/SVD ratio tended to increase with DR progression and was significantly correlated with DR severity, although the correlation was relatively weak. When both a reduction in GC-IPL thickness and deterioration of SVD are present as DR progresses, a significant increase in the GC-IPL/SVD ratio indicates greater impairment of SVD than GC-IPL thinning. Considering these results, impairment of the retinal vasculature may precede inner retinal layer thinning as DR progresses. Although further prospective studies are needed to validate this hypothesis, impaired retinal perfusion would precede damage to the neural tissue of the retina, which would then cause changes in the structure of the inner retinal layer.

DRN is known to occur in the relatively early stage of DR, and can be observed before clinical DR changes in fundus phtography.[4,6–8,16,25] Sohn et al.[26] reported that the inner retinal layer was significantly thinner in eyes with DM than control eyes, although retinal capillary density did not differ between the two groups; this indicates that DRN precedes microvascular changes in DR; however, the retinal capillary density was evaluated in only 12 donor eyes (6 from deceased donors with DM and no or minimal DR, and 6 from deceased donors without DM) using a biotinylated UEA-1-stained image. After the development of OCTA, which allows for visualization of fine retinal microvasculature as well as quantitative perfusion measurements, many studies have reported impairment of retinal microvasculature in patients with DM without clinical DR.[7,14,25,27,28] Therefore, it cannot be concluded that DRN precedes microvascular impairment; rather, microvascular impairment would thin the inner retinal layer, as discussed earlier.

Our study had several limitations. First, the retrospective nature of the work inevitably introduced some selection bias. Second, we could not analyze the parameters of deep vascular complex, as the automatic AngioPlex microcirculation parameters pertain only to the superficial vascular plexus. The main strength of our study was that it is the first to reveal the difference between the degree of inner retinal layer damage and microvasculature damage according to DR progression. Additionally, we included OCTA images with signal strength $\geq$9 for accurate analyses.

In conclusion, patients with DM had a thinner GC-IPL and lower macular SVD compared to healthy individuals. As the DR stage progressed, GC-IPL thickness tended to decrease and macular SVD showed a significant decrease. Therefore, OCTA appears to be more sensitive to retinal vasculature damage than fluorescein angiography in the early stage of DR, and is also useful for monitoring vascular damage, which worsens as DR progresses. Additionally, the GC-IPL/SVD ratio showed a significant increase as DR progressed, and the DR stage was significantly correlated with the GC-IPL/SVD ratio. The impairment of retinal vasculature was more prominent than GC-IPL thinning as DR progressed, which supports the premise that the impairment of retinal vasculature precedes DRN.

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