Diabetic retinopathy (DR) is a major complication of diabetes mellitus (DM), with 93 million cases being estimated globally. DR, which is the leading cause of blindness in the working-age population, causes damage to the retina of various forms. 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. These two types of damage are known to be related to each other. Inner retinal reduction can cause microvascular impairment, and impaired retinal perfusion can lead to inner retinal atrophy. Therefore, a better understanding of the relationship between the GC-IPL and vessel density of superficial vascular plexus (SVD) ratio was 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, 81.35 ± 6.74, and 79.89 ± 9.16 μm in each group, respectively (P < 0.001). The mean SVDs were 20.40 ± 1.26, 19.70 ± 1.56, 18.86 ± 2.04, and 17.82 ± 2.04 mm 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
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. 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 AngloPlex 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 center scan area of 3 × 3 mm pattern, and en face OCTA images were generated automatically by the OMAG algorithm of AngloPlex 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 mm² 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 ± 1.03, 7.69 ± 1.01, and 7.63 ± 0.102, respectively (P = 0.056). The demographic and clinical characteristics of the subjects are summarized in Table 1.
**TABLE 2.** Comparison of the Ganglion Cell-Inner Plexiform Layer Thickness Among Groups

| Group     | 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 ± 6.05  | 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     | 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 mm² 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).
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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{4}\textsuperscript{8}\textsuperscript{13} 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.\textsuperscript{16}\textsuperscript{18}\textsuperscript{21} Therefore, besides the impairment in visual function caused by neural tissue damage, DRN can also affect DR progression.\textsuperscript{22} Meanwhile, DRN is associated with other neurodegenerative diseases, especially Alzheimer's disease.\textsuperscript{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.\textsuperscript{8}\textsuperscript{23} As such, GC-IPL thickness could be a useful biomarker not only of DR progression but also the risk of Alzheimer's disease.\textsuperscript{23}

**DISCUSSION**

In this study, we evaluated GC-IPL thickness and retinal superficial vascular plexus using OCTA, according to DR severity. 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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{4}\textsuperscript{8}\textsuperscript{13} 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.\textsuperscript{16}\textsuperscript{18}\textsuperscript{21} Therefore, besides the impairment in visual function caused by neural tissue damage, DRN can also affect DR progression.\textsuperscript{22} Meanwhile, DRN is associated with other neurodegenerative diseases, especially Alzheimer's disease.\textsuperscript{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.\textsuperscript{8}\textsuperscript{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.\textsuperscript{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

\[\text{coeff} = 0.301; \ P < 0.001\]
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 SPD may be a more sensitive parameter to identify the changes in vascular flow than SVD 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. 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 microvascular impairment in DR.

Lee et al. reported that GC-IPL thickness was significantly associated with macular ED in patients with type 2 diabetes. Vujosevic et al. also found significant correlations between OCTA parameters, including VA 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. 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 photography. Sohn et al. reported that the DR, and can be observed before clinical DR changes in fundus photography. Sohn et al. reported that the inner retinal layer damage and microvasculature damage according to DR progression. Additionally, we included OCTA images with signal strength ≥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.

Acknowledgments

Author Contributions: Design and conduct of the study: M.W.L. and J.Y.K. Collection of data: C.K.R., H.Y.Y., and J.Y.K. Analysis and interpretation of data: M.W.L., J.Y.S., H.B.L., and J.Y.K. Writing the article: M.W.L., J.Y.S., and J.Y.K. Critical revision of the article: M.W.L., H.B.L., and J.Y.K. Final approval of the article: J.Y.S., M.W.L., C.K.R., H.B.L., H.Y.Y., and J.Y.K.

Disclosure: J.-Y. Sung, None; M.-W. Lee, None; H.-B. Lim, None; C.-K. Ryu, None; H.-Y. Yu, None; J.-Y. Kim, None

References

1. Yau JW, Rogers SL, Kawasaki R, et al. Global prevalence and major risk factors of diabetic retinopathy. Diabetes Care. 2012;35(3):556–564.
2. Klein R, Klein B, Moss S, et al. Prevalence and risk of diabetic retinopathy: does it really matter? Diabetologia. 2019;137(10):1125–1132.
3. Lee M-W, Lee W-H, Ryu C-K, et al. Effects of prolonged type 2 diabetes on the inner retinal layer and macular microvasculature: an optical coherence tomography angiography study. J Clin Med. 2020;9(6):1849.
4. Sinó R, Hernández C. Neurodegeneration in the diabetic eye: new insights and therapeutic perspectives. Trends Endocrinol Metab. 2014;25(1):23–33.
5. Brownlee M. Biochemistry and molecular cell biology of diabetic complications. Nature. 2001;414(6865):813–820.
6. Lim HB, Shin YI, Lee MW, et al. Longitudinal changes in the peripapillary retinal nerve fiber layer thickness of patients with type 2 diabetes. JAMA Ophthalmol. 2019;137(10):1125–1132.
7. Lee M-W, Lee W-H, Ryu C-K, et al. Effects of prolonged type 2 diabetes on the inner retinal layer and macular microvasculature: an optical coherence tomography angiography study. J Clin Med. 2020;9(6):1849.
8. Sinó R, Stitt AW, Gardner TW. Neurodegeneration in diabetic retinopathy: does it really matter? Diabetologia. 2018;61(9):1902–1912.
9. Ng DS, Chiang PP, Tan G, et al. Retinal ganglion cell neuronal damage in diabetics and diabetic retinopathy. Clin Exp Ophthalmol. 2010;44(4):245–250.
10. Arend O, Wolf S, Jung F, et al. Retinal microcirculation in patients with diabetes mellitus: dynamic and morphological analysis of perifoveal capillary network. Br J Ophthalmol. 1991;75(9):514–518.

11. Sinclair SH. Macular retinal capillary hemodynamics in diabetic patients. Ophthalmology. 1991;98(9):1580–1586.

12. Cao D, Yang D, Huang Z, et al. Optical coherence tomography angiography discerns preclinical diabetic retinopathy in eyes of patients with type 2 diabetes without clinical diabetic retinopathy. Acta Diabetologica. 2018;55(5):469–477.

13. Nesper PL, Roberts PK, Onishi AC, et al. Quantifying microvascular abnormalities with increasing severity of diabetic retinopathy using optical coherence tomography angiography. Invest Ophthalmol Vis Sci. 2017;58(6):BIO307–BIO315.

14. Vujosevic S, Muraca A, Gatti V, et al. Peripapillary microvascular and neural changes in diabetes mellitus: an OCT-angiography study. Invest Ophthalmol Vis Sci. 2018;59(12):5074–5081.

15. Wilkinson C, Ferris FL, III, Klein RE, et al. Proposed international clinical diabetic retinopathy and diabetic macular edema disease severity scales. Ophthalmology. 2003;110(9):1677–1682.

16. Lim HB, Shin YI, Lee MW, et al. Ganglion Cell–Inner Plexiform Layer Damage in Diabetic Patients: 3-Year Prospective, Longitudinal, Observational Study. Sci Rep. 2020;10(1):1–9.

17. Carrasco E, Hernández C, Miralles A, et al. Lower somatostatin expression is an early event in diabetic retinopathy and is associated with retinal neurodegeneration. Diabetes Care. 2007;30(11):2902–2908.

18. Kusari J, Zhou S, Padillo E, et al. Effect of memantine on neuroretinal function and retinal vascular changes of streptozotocin-induced diabetic rats. Invest Ophthalmol Vis Sci. 2007;48(11):5152–5159.

19. Silva KC, Rosales MA, Biswas SK, et al. Diabetic retinal neurodegeneration is associated with mitochondrial oxidative stress and is improved by an angiotensin receptor blocker in a model combining hypertension and diabetes. Diabetes. 2009;58(6):1382–1390.

20. Luu CD, Szental JA, Lee S-Y, et al. Correlation between retinal oscillatory potentials and retinal vascular caliber in type 2 diabetes. Invest Ophthalmol Vis Sci. 2010;51(1):482–486.

21. Murata T, Nakagawa K, Khalil A, et al. The relation between expression of vascular endothelial growth factor and breakdown of the blood-retinal barrier in diabetic rat retinas. Lab Investigation; J Tech Meth Pathol. 1996;74(4):819–825.

22. Simão S, Costa MÁ, Sun JK, et al. Development of a normative database for multifocal electroretinography in the context of a multicenter clinical trial. Ophthalmic Res. 2017;57(2):107–117.

23. Simó R, Ciudin A, Simó-Servat O, Hernández C. Cognitive impairment and dementia: a new emerging complication of type 2 diabetes—the diabetologist’s perspective. Acta Diabetologica. 2017;54(5):417–424.

24. Bucolo C, Barbieri A, Viganò I, et al. Short-and Long-Term Expression of Vegf: A Temporal Regulation of a Key Factor in Diabetic Retinopathy. Front Pharmacol. 2021;12:707909.

25. Lee M-W, Lee W-H, Ryu C-K, et al. Peripapillary Retinal Nerve Fiber Layer and Microvasculature in Prolonged Type 2 Diabetes Patients Without Clinical Diabetic Retinopathy. Invest Ophthalmol Vis Sci. 2021;62(2):9.

26. Sohn EH, van Dijk HW, Jiao C, et al. Retinal neurodegeneration may precede microvascular changes characteristic of diabetic retinopathy in diabetes mellitus. Proc Natl Acad Sci. 2016;113(19):E2655–E2664.

27. Zeng Y, Cao D, Yu H, et al. Early retinal neurovascular impairment in patients with diabetes without clinically detectable retinopathy. Br J Ophthalmol. 2019;103(12):1747–1752.

28. Dimitrova G, Chihara E, Takahashi H, et al. Quantitative retinal optical coherence tomography angiography in patients with diabetes without diabetic retinopathy. Invest Ophthalmol Vis Sci. 2017;58(1):190–196.