Correlation Between Ganglion Cell Layer Thinning and Poor Visual Function After Resolution of Diabetic Macular Edema

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Purpose. To assess the thickness of the ganglion cell-inner plexiform layer (GCIPL) in eyes with resolved diabetic macular edema (DME), using spectral-domain optical coherence tomography (SD-OCT), and its relationship with the visual function.

Methods. This retrospective observational case-control cohort study included eyes of diabetic patients with resolved DME (r-DME eyes), that is, normal central macular thickness (CMT) after treatment of DME, and eyes of aged-matched diabetic patients without maculopathy (no-DME eyes). The GCIPL thickness was measured on a macular cube SD-OCT scan using a specific automatic segmentation algorithm. Linear regression analyses were performed to determine the association between the GCIPL thickness and the visual acuity (VA) measured at the time of the OCT measurement.

Results. Average GCIPL thickness was reduced in r-DME eyes compared with no-DME eyes (74 ± 14 μm versus 83.2 ± 6 μm, $P = 0.0189$), whereas no significant difference in mean CMT was observed (260.0 ± 34 μm versus 265.7 ± 22 μm, $P = 0.847$). Visual acuity significantly correlated with the average GCIPL thickness ($r = 0.8$, $P < 0.0001$) and minimum GCIPL thickness ($r = 0.84$, $P < 0.0001$) in r-DME eyes.

Conclusions. Despite favorable anatomic response and restoration of a CMT in the range of normal values after resolution of DME, the GCIPL thickness in r-DME eyes was lower than that in no-DME eyes and correlated with the VA. These results suggest that inner retinal alterations occurring in patients with DME and diabetic retinopathy may lead to visual deficiency persisting after treatment.

Keywords: diabetic macular edema, ganglion cell layer, optical coherence tomography

The correlation between the central macular thickness (CMT) and the visual acuity (VA) is low in diabetic macular edema (DME).[1] This is also observed after treatment: when intravitreal injections are effective in improving or normalizing the macular thickness of eyes with DME, even with normalized CMT, the VA does not systematically return to normal. The current paradigm relies on the identification of retinal damages responsible for visual impairment in DME. Most studies have focused on damages of outer retinal layers induced by DME, reporting associations between the loss of the inner segment-outer segment junction or external plexiform layer and the poor visual prognosis.[2–4] Fewer studies have investigated inner retinal changes in DME,[5] and particularly, at the retinal ganglion cell (RGC) layer. Pelosini et al.[5] studied the amount of tissue located between the two plexiform layers on "en face" OCT scans, and showed that the retinal tissue integrity may be used as an indicator of visual function before treatment.

Although the reliable identification of retinal structures on optical coherence tomography (OCT) images in the presence of retinal swelling remains difficult, assessing retinal layers on a "dried" retina after resolution of DME seems a valuable alternative.

The quantitative measurement of the ganglion cell–inner plexiform layer (GCIPL) thickness by spectral-domain OCT (SD-OCT) is now available, and is especially used in glaucoma studies.[6,7] This automatic method allows reliably and objectively assessing GCIPL changes in the macula.

The aim of this study was to describe quantitative changes of the inner retina in eyes with resolved DME (r-DME) and in control diabetic eyes without DME (no-DME eyes), using the automatic OCT-based GCIPL measurement and to correlate these measurements with the VA.

Materials and Methods

In this retrospective study conducted in a tertiary eye care center (Lariboisière Hospital, Paris, France), records of consecutive diabetic patients with DME seen in the center and treated with intravitreal injections (anti-VEGF or steroids) over a 1-year period (June 2012–July 2013) were reviewed. Inclusion criteria were as follows: age older than 18 years, dried retina after treatment of DME, imaged with Cirrus HD-OCT (Carl Zeiss Meditec, Dublin, CA, USA), with a CMT less than 315 μm, corresponding to normal value + 2 SDs: 277 + (2 × 19) μm,[8] associated with restoration of foveal pit, and with normal thickness of six Early Treatment Diabetic Retinopathy Study (ETDRS) surrounding area.

Exclusion criteria were the following: any non–diabetes-related ophthalmological or neurological disease that could affect or could have affected the VA or visual field (amblyopia,

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history of papilledema or glaucoma); ischemic maculopathy; recent panretinal photocoagulation (PRP) (<6 months); absence of ETDRS VA assessment; pathologic myopia (defined as myopia >6 diopters or fundus abnormalities); OCT scanning of poor quality, defined as scans with a signal strength less than 6 (maximum 10), not centered on the fovea, lack of uniform brightness, retinal nerve fiber layer (RNFL) discontinuity or misalignment, involuntary saccade or blinking artifacts, or presence of algorithm segmentation failure. Absence of segmentation errors or potential automated artifacts was manually checked for all scans included in the study. All data were assessed by a chart review, including sex, age, diabetes type and duration, serum glycated hemoglobin level (HbA1c), ETDRS best-corrected VA after refraction, IOP and previous ophthalmologic treatments (panretinal or macular grid photocoagulation, intravitreal injections). Fluorescein angiography was reviewed to confirm absence of ischemic maculopathy (defined by an enlargement of the foveal avascular zone >1000 μm in at least one diameter). The OCT images used to assess macular resolution were analyzed either 1 month after third ranibizumab intravitreal injection, or 2 months after dexamethasone intravitreal injection.

The control group (no-DME eyes) consisted of consecutive age-matched diabetic patients from an observational cohort followed during the same period in the same department, and with minimal or no diabetic retinopathy (DR) without any history of diabetic maculopathy, laser treatment, or intravitreal injections. Controls with an underlying comorbid ocular condition were excluded.

This study was approved by the Ethics Committee of the French Society of Ophthalmology (IRB 00008855 Societe Française d'Ophtalmologie IRB#1) and met the tenets of the Declaration of Helsinki. Informed consent was obtained for all patients.

Optical Coherence Tomography

According to current practice, OCT scanning protocol was performed as follows, after pupil dilation, using the Cirrus high-resolution SD-OCT system: the macular cube protocol included macular thickness analysis and ganglion cell layer analysis (GCA). The GCA algorithm was included in the Cirrus HD-OCT 6.0 software. It detected and measured the macular GCIPL thickness within a $6 \times 6 \times 2$-mm elliptical annulus area centered on the fovea. The algorithm has been described previously in detail.[9] The size of the inner ring of the annulus was chosen to exclude the foveal area (vertical radius: 0.5 mm, horizontal radius: 0.6 mm), where the ganglion cell layer (GCL) is thin and difficult to detect. However, the dimension of the outer ring was selected to conform closely to the real anatomy of the macular region, where the GCL is the thickest in a healthy eye. The GCA reports the average GCIPL thickness over six sectorial areas (superotemporal, superior, superonasal, inferonasal, inferior, and inferotemporal) that form an elliptical annulus around the fovea, as well as the overall average for the annulus. The GCA also reports the minimum GCIPL thickness, which is the lowest GCIPL thickness over a single meridian crossing the annulus.

Visual Function

Standard, high-contrast VA was assessed using ETDRS charts at 3.2 m as part of the standard follow-up of diabetic patients.

Statistical Analyses

Statistical analyses were performed using GraphPad Prism 5 (GraphPad Software, San Diego, CA, USA). Results are expressed as the mean ± SD. Continuous data were analyzed using the Mann-Whitney test for populations and the Fisher's exact test was used to compare qualitative data. The Spearman test and simple linear regression analysis were used to test correlations between variables; P less than 0.05 was considered significant.

Table 1. Baseline Characteristics of Studied Eyes

	No-DME Eyes	r-DME Eyes	*P*
Eyes	21	21	
Age, y	60 ± 8.6	62 ± 8.8	0.497*
Sex, male/female	12/4	14/6	1.0†
VA, ETDRS letters	86.9 ± 3.4	64.4 ± 11.5	<0.0001
Pseudophakic status	0	12 (57%)	0.0002†
Duration of diabetes, y	12.2 ± 6.4	19.9 ± 13.3	0.0667*
HbA1c, %	7.8 ± 1	7.2 ± 0.8	0.059*
Diabetes type, 1/2	0/21	2/19	0.049†
Proliferative diabetic retinopathy stabilized after PRP	0	14 (67%)	<0.0001†
Duration of DME, mo	N/A	33 ± 18	

Values are presented as the mean ± SD.
* Mann-Whitney test.
† Fisher's exact test.

Results

Subjects

Twenty-one eyes of 19 diabetic patients with resolved DME after treatment for macular edema (r-DME eyes, defined by CMT <315 μm) and 21 eyes of 16 diabetic patients without maculopathy (no-DME eyes) were included in the study. Baseline characteristics are shown in Table 1. Among the r-DME eyes, 13 (62%) had been previously treated with ranibizumab, including seven eyes treated with combined focal laser and ranibizumab, and eight (38%) had previously received dexamethasone intravitreal implant (Dex-I), including three eyes treated with combined focal laser and Dex-I. Fourteen eyes (67%) had previously received PRP and the remaining eyes had nonproliferative DR.

All 21 of the no-DME eyes were phakic, and DR was mild or absent.

No significant difference was found between r-DME and no-DME eyes for age ($P = 0.497$), sex ($P = 1.0$), and HbA1c ($P = 0.059$) (Table 1).

Assessment of the GCIPL Thickness in r-DME and No-DME Eyes

Table 2 shows the mean CMT and average GCIPL thickness in r-DME and no-DME eyes. Although no significant difference in mean CMT between both groups was observed, the average GCIPL thickness of the retina was significantly reduced in r-DME eyes: 73.8 ± 14.3 μm versus 83.2 ± 5.9 μm in no-DME eyes, $P = 0.0189$. Moreover, a significant difference was found for the minimum GCIPL thickness: 57 ± 22 μm in r-DME eyes and 81 ± 5 μm in no-DME eyes, $P = 0.0001$. Of the six macular sectors, the superotemporal sector had the thickest value, whereas the inferior sector had the thinnest GCIPL in r-DME eyes. The assessment of the outer retinal thickness (full retinal thickness – GCIPL thickness) showed no significant difference between r-DME and no-DME eyes (186 ± 35 μm versus 183 ± 23 μm, $P = 0.5208$).

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Table 2. Central Macular Thickness and Distribution of Macular GCIPL Thickness in No-DME and r-DME Eyes

	Mean (95% CI), μm		
SD-OCT Thicknesses	**No-DME Eyes**	**r-DME Eyes**	***P***
CMT	265.7 (255.8–275.6)	260.0 (244.3–275.6)	0.847
Average GCIPL	83.2 (80.5–85.9)	73.8 (67.3–80.3)	0.0189*
Minimum GCIPL	81.2 (78.7–83.7)	57.3 (47.4–67.2)	<0.0001*
Superotemporal GCIPL	84.1 (81.7–86.4)	75.6 (66–85.1)	0.1859*
Superior GCIPL	83.5 (80.8–86.1)	72.4 (64.1–80.7)	0.026*
Superonasal GCIPL	84.0 (80.9–87.0)	73.5 (64.2–82.8)	0.079*
Inferonasal GCIPL	82.7 (79.3–86.1)	72.1 (65.4–78.8)	0.0287*
Inferior GCIPL	81.9 (78.5–85.3)	70.2 (63.6–76.8)	0.009*
Inferotemporal GCIPL	84.5 (81.6–87.4)	75.3 (66.4–84.2)	0.0094*

Data represent the thicknesses in micrometers. CI, confidence interval.
* Mann-Whitney test.

Among the r-DME eyes, no significant difference in CMT or average and minimum GCIPL thicknesses was observed between eyes with and without previous PRP (Table 3). No significant difference in average and minimum GCIPL thicknesses was found between eyes with and without previous focal laser (Table 3).

Relationship Between VA and OCT Measurements

In r-DME eyes, the VA significantly correlated with the average GCIPL thickness ($r = 0.804$; $P < 0.0001$) and minimum GCIPL thickness ($r = 0.835$; $P < 0.0001$), whereas no correlation was found between the VA and the CMT ($r = 0.18$; $P = 0.45$). The Figure illustrates the simple linear regression between the minimum GCIPL thickness and the VA (Fig. A) and between the average GCIPL thickness and the VA (Fig. B) in r-DME eyes.

Discussion

This study showed that GCIPL thicknesses were thinner in eyes with r-DME than in diabetic eyes without maculopathy, and that the average and minimum GCIPL thicknesses strongly correlated with the VA.

The quantitative assessment of macular structures is difficult to perform in case of macular swelling, and the originality of this work relied on the analysis of inner retina changes on a "dried" retina. This method avoids segmentation errors made by the ganglion cell analyzer, and allows reliably, automatically and objectively assessing GCIPL changes in the macula. This algorithm has been previously used and approved to detect local ganglion cell loss in early glaucoma.[10] Measurements of average GCIPL thicknesses in this study seem reliable, as they were comparable to those found in the literature: 82.7 μm (80.2–85.2 μm) in healthy eyes[10] and 83.2 (80.5–85.9 μm) in our series of no-DME eyes. Interestingly, this study supports that the minimum GCIPL thickness on the radius showed a better ability to discriminate healthy eyes from early glaucomatous eyes than did the average GCIPL thickness.[10] Our results are consistent with these findings. In this study, the high correlation coefficient found between the minimum GCIPL thickness and the VA ($r = 0.84$) could be used as a valuable index of visual function. Because the GCA algorithm reports GCIPL thicknesses averaged along each radius coming out of the fovea, the minimum GCIPL thickness reflects an extreme value, of a very thin sector, particularly sensitive to cell loss. This value could act as a compass to indicate the location of local RGC loss in eyes with diabetic maculopathy. This approach is not dependent on the average thickness of an area. Calculating the average thickness in a particular sector underestimates the local RGC loss in sectors in which there are normal or less affected areas, or persistent edema. This high correlation supports the hypothesis that the minimum GCIPL thickness is more accurate for detecting the RGC loss in diabetic maculopathy.

Although an association between outer retina abnormalities and the VA has been reported previously,[11] to our knowledge, the correlation between inner retinal layers and the VA in diabetic eyes with maculopathy has not been studied. Pelosini et al.[5] reported an interesting correlation between the VA at the time of the edema and the amount of tissue located between the two plexiform layers on "en face" OCT scans, and have shown that the retinal tissue integrity could be an

Table 3. Central Macular Thickness, and Average and Minimum GCIPL Thicknesses According to Previous Focal Laser or PRP in r-DME Eyes

	Average GCIPL Thickness			Minimum GCIPL Thickness			CMT		
	No Focal Laser	**Focal Laser**	***P***	**No Focal Laser**	**Focal Laser**	***P***	**No Focal Laser**	**Focal Laser**	***P***
No. of eyes	10	11		10	11		10	11	
Mean, μm	75.1	72.6	0.288*	60.3	54.55	0.499*	244.5	274	0.049*
SD, μm	17.8	11		24.34	19.78		38.4	24.3	
	No PRP	**PRP**	***P***	**No PRP**	**PRP**	***P***	**No PRP**	**PRP**	***P***
No. of values	7	14		7	14		7	14	
Mean, μm	70.9	75.3	0.956*	63	55	0.240*	258.7	260.6	0.675*
SD, μm	13.41	14.97		23.6	21.3		49.02	26.75	

* Mann-Whitney test.

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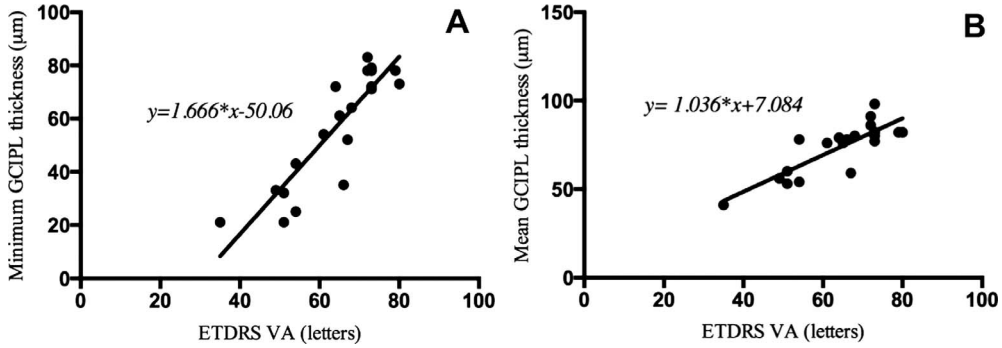

FIGURE. Scatter plot of simple linear regression between ETDRS VA and Minimum GCIPL thickness (**A**) and between ETDRS VA and Average GCIPL thickness (**B**), in r-DME eyes ($n = 21$): interestingly, the five eyes with the best VAs (from 73 to 80 ETDRS letters) had all a minimum GCIPL thickness thicker than 71 µm and the five eyes with the worst VAs (from 35 to 54 ETDRS letters) had a minimum GCIPL thickness thinner than 43 µm.

indicator of visual function before treatment. Our results highlight an interesting correlation between the GCIPL thickness and the VA, but do not imply a causative effect. Actually, although damages in the outer retinal layers were not assessed in this study, they could also contribute to the decrease in VA after resolution of DME. Of note, as mentioned in the Results section, outer retinal layer thicknesses were not significantly different between both groups.

Reasons for GCIPL thinning may be multiple, including vascular changes and primary neuronal degeneration. Intraretinal fluid accumulation by itself induces Müller cell ballooning and retinal degeneration. In addition, a diabetes-induced retinal neurodegeneration starts early after the onset of diabetes, even in the absence of notable vascular changes on fundus analysis, and could contribute to capillary degeneration.[12] In a study conducted in patients with type 2 diabetes with minimal DR without history of DME,[13] the mean GCIPL thickness measured using another method was 79.1 ± 11.1 µm, showing an early decrease in inner retinal layer thickness. Severe ischemia also could increase the ganglion cell degeneration, which is more severe in patients with more severe DR than in patients with mild retinopathy, as previously reported in animal models.[14,15] The GCIPL thinning also could reflect previous poor glycemic control, rather than a recently balanced diabetes with HbA1c levels in r-DME eyes. Finally, potential toxic effects of intravitreal treatments on ganglion cells could occur. In this study, most eyes had previous history of many pharmacological treatments (steroids, anti-VEGF). It has been recently reported that ranibizumab suppresses the autocrine VEGF-induced survival of purified RGCs (Froger N, et al. *IOVS* 2014;55:ARVO E-Abstract 2391). Regarding the effects of previous laser treatment, in patients with previous macular photocoagulation, laser burns also could have reduced the GCIPL thickness in some sectors, affecting particularly the minimum GCIPL thickness. However, average and minimum GCIPL thicknesses according to previous focal laser or PRP in r-DME eyes were not different, and the small number of eyes does not allow further exploring this point. Transitory increases in macular GCIPL and peripapillary RNFL thicknesses throughout the 1-year post-PRP follow-up were found in a prospective, interventional case series study of 35 eyes.[16] These data could be explained by PRP-induced retinal inflammation and edema in the early post-PRP phase, secondary to early glial cell activation. In our study, all patients had received previous PRP more than 6 months before, so that should not have affected our results.

Despite favorable anatomic response and restoration of a CMT in the range of normal values after resolution of DME, the GCIPL thickness was lower than normal and correlated with the VA. These results suggest that inner retinal alterations could be associated with DME and visual deficiency persisting even after treatment. Assessing the GCIPL thickness at the time of the edema and using it as a prognostic factor for visual recovery could be an interesting next step to investigate.

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References

1. Diabetic Retinopathy Clinical Research Network, Browning DJ, Glassman AR, et al. Relationship between optical coherence tomography-measured central retinal thickness and visual acuity in diabetic macular edema. *Ophthalmology*. 2007;114:525–536.
2. Alasil T, Keane PA, Updike JF, et al. Relationship between optical coherence tomography retinal parameters and visual acuity in diabetic macular edema. *Ophthalmology*. 2010;117:2379–2386.
3. Uji A, Murakami T, Nishijima K, et al. Association between hyperreflective foci in the outer retina, status of photoreceptor layer, and visual acuity in diabetic macular edema. *Am J Ophthalmol*. 2012;153:710–717.e1.
4. Sakamoto A, Nishijima K, Kita M, Oh H, Tsujikawa A, Yoshimura N. Association between foveal photoreceptor status and visual acuity after resolution of diabetic macular edema by pars plana vitrectomy. *Graefes Arch Clin Exp Ophthalmol*. 2009;247:1325–1330.
5. Pelosini L, Hull CC, Boyce JF, McHugh D, Stanford MR, Marshall J. Optical coherence tomography may be used to predict visual acuity in patients with macular edema. *Invest Ophthalmol Vis Sci*. 2011;52:2741–2748.
6. Choi YJ, Jeoung JW, Park KH, Kim DM. Glaucoma detection ability of ganglion cell-inner plexiform layer thickness by spectral-domain optical coherence tomography in high myopia. *Invest Ophthalmol Vis Sci*. 2013;54:2296–2304.

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7. Jeoung JW, Choi YJ, Park KH, Kim DM. Macular ganglion cell imaging study: glaucoma diagnostic accuracy of spectral-domain optical coherence tomography. *Invest Ophthalmol Vis Sci*. 2013;54:4422–4429.
8. Wolf-Schnurrbusch UEK, Ceklic L, Brinkmann CK, et al. Macular thickness measurements in healthy eyes using six different optical coherence tomography instruments. *Invest Ophthalmol Vis Sci*. 2009;50:3432–3437.
9. Mwanza J-C, Durbin MK, Budenz DL, et al. Profile and predictors of normal ganglion cell-inner plexiform layer thickness measured with frequency-domain optical coherence tomography. *Invest Ophthalmol Vis Sci*. 2011;52:7872–7879.
10. Takayama K, Hangai M, Durbin M, et al. A novel method to detect local ganglion cell loss in early glaucoma using spectral-domain optical coherence tomography. *Invest Ophthalmol Vis Sci*. 2012;53:6904–6913.
11. Murakami T, Nishijima K, Akagi T, et al. Segmentational analysis of retinal thickness after vitrectomy in diabetic macular edema. *Invest Ophthalmol Vis Sci*. 2012;53:6668–6674.
12. Barber AJ, Lieth E, Khin SA, Antonetti DA, Buchanan AG, Gardner TW. Neural apoptosis in the retina during experimental and human diabetes. Early onset and effect of insulin. *J Clin Invest*. 1998;102:783–791.
13. van Dijk HW, Verbraak FD, Kok PHB, et al. Early neurodegeneration in the retina of type 2 diabetic patients. *Invest Ophthalmol Vis Sci*. 2012;53:2715–2719.
14. Howell SJ, Mekhail MN, Azem R, Ward NL, Kern TS. Degeneration of retinal ganglion cells in diabetic dogs and mice: relationship to glycemic control and retinal capillary degeneration. *Mol Vis*. 2013;19:1413–1421.
15. Schnichels S, Hagemann U, Januschowski K, et al. Comparative toxicity and proliferation testing of aflibercept, bevacizumab and ranibizumab on different ocular cells. *Br J Ophthalmol*. 2013;97:917–923.
16. Kim JJ, Im JC, Shin JP, Kim IT, Park DH. One-year follow-up of macular ganglion cell layer and peripapillary retinal nerve fibre layer thickness changes after panretinal photocoagulation. *Br J Ophthalmol*. 2014;98:213–217.

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