Disorganization of Retinal Inner Layers (DRIL) and Neuroretinal Dysfunction in Early Diabetic Retinopathy

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PURPOSE. To elucidate the relationship between disorganization of retinal inner layers (DRILs) and retinal function in diabetic patients without diabetic retinopathy (DR) and with nonproliferative DR, but without diabetic macular edema (DME).

METHODS. Fifty-seven participants with diabetes mellitus (DM) and 18 healthy controls underwent comprehensive ophthalmic examination, fundus photography, and spectral-domain optical coherence tomography. Scans of the fovea were evaluated for the presence of DRIL. Retinal function was evaluated using Early Treatment Diabetic Retinopathy Study (ETDRS) visual acuity, the quick contrast sensitivity function (qCSF) on the AST Sento Platform, short-wavelength automated perimetry (SWAP), standard automated perimetry (SAP), and frequency doubling perimetry (FDP). ANOVA and Kruskal-Wallis were used to compare retinal function in subjects with and without DRIL. Tukey-Kramer test and Wilcoxon were used for post hoc analysis.

RESULTS. DRIL was identified in 9 of 57 diabetic subjects. DRIL subjects had higher body mass index and longer diabetes duration compared to diabetic subjects without DRIL (P = 0.03 and P = 0.009, respectively). Subjects with DRIL had reduced ETDRS visual acuity (P = 0.003), contrast sensitivity function (P = 0.0003), and SAP performance (PSD, P < 0.0001) compared to controls and diabetic subjects without DRIL. Structural analysis revealed inner retinal thinning, and some outer retinal thinning, associated with DRIL.

CONCLUSIONS. Diabetic subjects with DRIL have reduced retinal function compared to those without DRIL, and defective retinal lamination may be an early cellular consequence of diabetes responsible for this in some patients. Following further longitudinal studies, DRIL may be a readily available and reliable structural biomarker for reduced retinal function in early diabetic neuroretinal disease.

Keywords: diabetic retinopathy, DRIL, OCT, contrast sensitivity, perimetry

Diabetic retinopathy (DR), a well-studied complication of diabetes mellitus (DM), affects approximately 35% of diabetic patients worldwide.1 DR affects the entire neurovascular unit of the retina,2,3 and recent studies have identified retinal dysfunction and structural changes resulting from neuroretinal disruption, sometimes occurring prior to the characteristic microvascular clinical findings of DR.4–10 With the prevalence of DM predicted to double by 2030,8 there is a crucial need to clarify the cellular mechanisms of neurovascular unit disruption, and to identify reliable endpoints based on these cellular defects. These steps may lead to improved means to develop treatments to prevent vision loss from DM.9

Until recently, the search for a structural marker of visual function in diabetic patients has been unsuccessful, because commonly measured parameters, such as optical coherence tomography (OCT) retinal thickness,11,12 are of limited predictive value for current and future visual acuity. However, investigators have used spectral-domain optical coherence tomography (SD-OCT) to explore disorganization of the retinal inner layers (DRILs) as a new potential marker.12–16 Retinal tissue has a very organized structure, and disturbance of its layers, especially in the macular region, can reduce visual function. Sun et al.12 showed that the presence of foveal DRIL was associated with worse baseline visual acuity in patients with diabetic macular edema (DME) and predicted visual acuity at 8 months. The presence of foveal DRIL was also associated with reduced visual acuity in patients with resolved DME.13,17 Nicholson et al.14 found that the presence of DRIL was correlated with macular capillary nonperfusion, a metric of maculopathy, in severe nonproliferative DR (NPDR) and proliferative DR. Also, Balaratnasingam et al.15 showed that the extent of DRIL correlated with the size of the foveal avascular zone, another metric of maculopathy, in patients with all grades of DR, without DME. Most recently, Das et al.16 found the presence of DRIL was associated with increasing severity of DR, specifically proliferative DR. They also found that DRIL was associated with structural disruption in the outer retina, specifically the ellipsoid zone (EZ) and the external limiting
membrane (ELM).\textsuperscript{16} Taken together, DRIL appears to be a readily available and reliable predictive tool for visual acuity, capillary perfusion, and other morphological changes in severe DR, proliferative DR, and DME.

Nevertheless, DRIL has not been studied in diabetic patients without clinical evidence of DR and with early-stage DR, and its role in early diabetic neuroretinal impairment remains unclear. Furthermore, its ability to serve as a biomarker for other relevant aspects of visual function, such as visual fields and contrast sensitivity, has not been determined. Thus, the purpose of this research was to serve as a pilot study to elucidate the effects of DRIL in early neuroretinal disease. Specifically, we were interested in determining the effects of DRIL on several aspects of visual function in patients without clinical evidence of DR and with DR, without DME or proliferative changes.

**METHODS**

This study was conducted at the University of Michigan W. K. Kellogg Eye Center. The protocol was approved by the Institutional Review Board of the University of Michigan Medical School (HUM 99155). Patients were recruited from the University of Michigan clinics and University of Michigan Health Research website between March 2016 and January 2017. Written informed consent was obtained from all patients before participation in the study.

**Subject Enrollment and Evaluation**

The study protocol and cohort were described previously.\textsuperscript{4} Subjects were enrolled into four groups: healthy subjects (control group), diabetics without retinopathy (no DR group), diabetics with mild NPDR (mild NPDR group), and diabetics with moderate to very severe NPDR (moderate NPDR group). Inclusion criteria for the control group were: (1) age $\geq$ 18 years, (2) no clinical diagnosis of diabetes, and (3) Early Treatment Diabetic Retinopathy Study (ETDRS) DR severity level of 10—no detectable retinopathy. Inclusion criteria for the diabetic group were: (1) age $\geq$ 18 years and (2) diabetes as defined by the American Diabetes Association criteria for diagnosis.\textsuperscript{18} The mild NPDR group included patients with ETDRS DR grade 20 to 35 and the moderate NPDR group included patients with ETDRS DR grade 43 to 53.

Exclusion criteria for all the groups were: any neurological or systemic disease (other than DM), any drug intake that could impair vision, Snellen or equivalent best corrected visual acuity (BCVA) worse than 20/40, spherical equivalent more than $\pm$ 6.0 diopters (D), proliferative DR, DME as judged by fundus photographs or OCT, pregnancy or nursing, and inability to give informed consent or to complete testing.

All subjects underwent a comprehensive ophthalmologic examination in the Kellogg Clinic Research Center, including slit lamp examination, applanation tonometry, measurement of BCVA with the electronic visual acuity (EVA) tester using the e-ETDRS protocol, color fundus photography, SD-OCT, contrast sensitivity using quick contrast sensitivity function (qCSF) method on the Sentio Platform (Adaptive Sensory Technology, San Diego, CA, USA),\textsuperscript{19} and three methods of perimeter testing: short-wavelength automated perimetry (SWAP), standard automated perimetry (SAP), and frequency doubling perimetry (FDP).

**Fundus Photography**

Color fundus photographs were taken using nonsimultaneous stereoscopic, on-axis, nonsteered, 200\degree ultrawide field (UWF) imaging (Optos 200TX, Optos plc, Dunfermline, UK), and the images were magnified to the equivalent field dimensions of 7-standard fields of the ETDRS scale. DR grade was determined based on the images and clinical evaluation by VMC and TWG.

**Spectral-Domain Optical Coherence Tomography**

SD-OCT (Spectralis HRA+OCT, Heidelberg Engineering, Inc., Heidelberg, Germany) was performed using the following scan acquisition parameters: macular scan volume, 37 B-scans, each spaced 120 mm, $15\times15\degree$, automatic real-time (ART) mean of 12 in high resolution (HR) mode. For quality control, all OCT scans were performed in a masked fashion by VMC and KAJ, with the same technique and parameters used for cases and controls.

Three SD-OCT scans above and below the foveal scan line were evaluated for the presence of DRIL in each subject by two reviewers (KAJ and CS) under masked conditions. DRIL was identified when the boundaries of the ganglion cell, inner plexiform layer, inner nuclear layer, and outer plexiform layer could not be identified and demarcated.\textsuperscript{12} The horizontal extent of DRIL was measured on each scan within the 6 mm ring of the ETDRS grid centered at the fovea. Pearson correlation coefficients were used to determine agreement of DRIL measurements among the two reviewers. On scans with $r < 0.8$, the DRIL horizontal length was re-measured until the calculations were agreeable. The total extent of DRIL for each subject was then calculated by taking the average measurements from both reviewers across all seven scans (foveal scan with three scans above and below). The retinal layers on each SD-OCT scan were also segmented semiautomatically using the built-in software of the Heidelberg Spectralis. The boundaries of all segmented layers were carefully reviewed by two reviewers (VMC and KAJ) and adjusted when necessary. Retinal thickness was analyzed using the ETDRS grid, which included the 1 mm central fovea, 3 mm inner ring, and 6 mm outer ring. The inner and outer rings were sectioned into superior, inferior, temporal, and nasal quadrants. The retinal thickness was recorded for the total retina, total inner retina, and total outer retina (between the ELM and RPE). The total inner retina thickness was used because we could not accurately segment the individual retinal layers in eyes with DRIL.

**ETDRS Visual Acuity**

Visual acuity was evaluated using the EVA Tester (Jaeb Center for Health Research, Tampa, FL, USA) with E-ETDRS protocol. One eye of each subject was selected for the study; if both eyes met eligibility criteria, the eye with more severe retinopathy was chosen. If both eyes were eligible for the same retinopathy group, then the eye with the better visual acuity was selected.

**Contrast Sensitivity**

Contrast sensitivity was evaluated using the Quick Contrast Sensitivity (qCSF)\textsuperscript{19} method on the AST Sentio Platform (Adaptive Sensory Technology, San Diego, CA, USA),\textsuperscript{20} a computerized method for evaluating the contrast thresholds over a wider range of contrast (0.002% to 100%) and spatial frequency (approximately 1 to 27 cycles per degree).\textsuperscript{11} All participants were tested monocularly following measurement of BCVA, while the untested eye was covered with a patch. The area under the logCSF (AULCSF), integrated from 1.5 to 18 cpd, served as a metric of contrast sensitivity function and was used for statistical analysis.
and with the Shapiro-Wilk test. ANOVA was used for parametric variables and the Kruskal-Wallis test was used to compare continuous nonparametric variables. The Tukey-Kramer HSD (parametric) and Wilcoxon (nonparametric) tests were used for post hoc analysis. The Bonferroni correction for multiple comparisons was applied to functional analyses such that the statistical significance would occur at $P \leq 0.005$. For other tests, $P \leq 0.05$ was considered statistically significant. Statistical analysis was performed with JMP, Version Pro 13 (SAS Institute, Inc., Cary, NC, 1989–2007).

**RESULTS**

**Demographics**

Table 1 describes the demographics of the 75 participants included in the study. ANOVA and Kruskal-Wallis tests were used to determine if there were differences among the groups. The cohort consisted of 68.4% males; the control group only included in the study. ANOVA and Kruskal-Wallis tests were used to determine if there were differences among the groups. Among the diabetic subjects, 78.9% had type 2 diabetes. Subjects with mild and moderate NPDR had a longer duration of diabetes than subjects with no DR ($P = 0.002$ and $P = 0.016$, respectively). Diabetic subjects had higher body mass index (BMI) and hemoglobin A1C (HbA1C) than control subjects ($P = 0.002$ for both). There was no difference in age or type of diabetes among the groups.

**Presence of DRIL**

The presence of DRIL was identified in SD-OCT scans from 9 of 57 (16%) of diabetic subjects and in none of the control subjects. Table 2 shows the demographic characteristics among all subjects based on the presence of DRIL. DRIL was identified in one diabetic subject without clinical evidence of DR, in four subjects with mild NPDR, and in four subjects with moderate-severe NPDR. The Figure shows an example of DRIL in a subject with moderate NPDR. t-tests and Fisher’s exact tests were used to determine if there were differences among the two groups in each retinopathy group. We found no significant differences in age, sex, diabetes type, diabetes

**Statistical Analysis**

Demographics data were summarized as means ± SDs for continuous variables, and frequencies for categorical variables. The data distribution was assessed for normality graphically

**Table 2.** Subject Demographics Based on DRIL Status

<table>
<thead>
<tr>
<th>DRIL status no DRIL/DRIL</th>
<th>Control ($n = 18$)</th>
<th>No DR ($n = 23$)</th>
<th>Mild NPDR ($n = 19$)</th>
<th>Moderate NPDR ($n = 15$)</th>
<th>$P$ Value, $n$</th>
<th>No DR/DRIL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y (SD)</td>
<td>51.7 (14.3)</td>
<td>53.7 (12.2)</td>
<td>57.4 (12.7)</td>
<td>59.9 (11.6)</td>
<td>0.25</td>
<td></td>
</tr>
<tr>
<td>Sex, male (%)</td>
<td>27.8</td>
<td>56.5</td>
<td>73.7</td>
<td>80</td>
<td>0.007*</td>
<td></td>
</tr>
<tr>
<td>Diabetes type, type 2 (%)</td>
<td>NA</td>
<td>78.4</td>
<td>68.4</td>
<td>93.3</td>
<td>0.21</td>
<td></td>
</tr>
<tr>
<td>Diabetes duration, y (SD)</td>
<td>NA</td>
<td>9.35 (8.67)</td>
<td>20.8 (12.5)</td>
<td>17.5 (11.4)</td>
<td>0.005*</td>
<td></td>
</tr>
<tr>
<td>BMI (SD)</td>
<td>26.2 (4.4)</td>
<td>32.9 (7.9)</td>
<td>30.4 (5.1)</td>
<td>35.7 (6.29)</td>
<td>0.001*</td>
<td></td>
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<tr>
<td>HbA1C, % (SD)</td>
<td>5.45 (0.26)</td>
<td>7.75 (1.99)</td>
<td>8.05 (1.0)</td>
<td>7.87 (1.54)</td>
<td>0.0005*</td>
<td></td>
</tr>
</tbody>
</table>

* Statistically significant result.
duration, BMI, and HbA1C among subjects with and without DRIL within each retinopathy group. When we did the same analysis, comparing subjects with and without DRIL among all diabetic subjects, we found that subjects with DRIL had higher BMI and longer diabetes duration than subjects without DRIL ($P = 0.03$ and $P = 0.009$, respectively).

**Horizontal Extent of DRIL**

The overall Pearson correlation coefficient for agreeable DRIL measurements was 0.98 between the two reviewers. The DRIL extent among the nine subjects ranged from 123.5 $\mu$m to 731.0 $\mu$m in the macular region, within the 6 mm ring of the ETDRS grid centered at the fovea. The least DRIL was identified in a 38-year-old female with 18 years of type 2 diabetes, and moderate NPDR. The greatest extent of DRIL was identified in a 73-year-old male, also with 18 years of type 2 diabetes and moderate NPDR (Fig.). The subject with DRIL but no clinical evidence of DR was a 41-year-old male with 12 years of type 2 diabetes. We compared the four subjects with <275 $\mu$m of DRIL (median extent of DRIL) to the five subjects with >300 $\mu$m of DRIL using Student’s t-tests and found no differences in age, diabetes duration, HbA1C, visual acuity, contrast sensitivity function, and perimetry performance. DRIL was identified in only one female subject (<275 $\mu$m DRIL) and only one subject with type 1 diabetes (<275 $\mu$m DRIL); the rest were type 2 diabetic males ($n = 7$).

**Retinal Function and DRIL**

Retinal function was evaluated using ETDRS visual acuity, the Quick Contrast Sensitivity method, SWAP, SAP, and FDP. Results are depicted in Table 3. ANOVA and Kruskal-Wallis tests were used to determine if there were differences among the groups and Bonferroni correction for multiple comparisons was applied such that statistical significance would occur at $P = 0.005$. Post hoc analysis was used to determine if there were differences among diabetic subjects with DRIL and without.

**OCT Thickness and DRIL**

Macular SD-OCT scans were obtained and segmented semiautomatically using the Heidelberg Spectralis software and two masked reviewers. The total retinal thickness, inner retinal thickness, and outer retinal thickness were compared among controls, diabetic subjects without DRIL, and diabetic subjects with DRIL. The Figure shows the result of the OCT analysis in the center, inner ring, and outer ring of the ETDRS grid. Diabetic subjects with DRIL had a 6% reduction in total retinal thickness compared to diabetic subjects without DRIL and control subjects both in the inferior and nasal quadrants of the inner ETDRS ring ($P = 0.03$ for both). In the same areas, diabetic subjects with DRIL had a 7% reduction in inner retinal thickness compared to diabetic subjects without DRIL and control subjects ($P = 0.05$ for both). This suggests that the thinning of the inner retina may be resulting in a significant thinning of the total retina. In the outer retina, diabetic subjects with DRIL had a 2.5% reduction in retinal thickness compared to diabetic subjects without DRIL and a 2% reduction compared to control subjects ($P = 0.05$) in the superior segment of the outer ETDRS grid. Taken together, DRIL appears to be associated to retinal thinning mostly in the inner retina, but also in the outer retina.

**DISCUSSION**

The identification of secondary tests and endpoints for screening and disease management is imperative for the
The development of new treatments to prevent the progressive vision loss associated with diabetic neuroretinal disease. Previous studies have shown that disruption of the inner retina, identified on SD-OCT as DRIL, can predict visual acuity in patients with vision threatening DR. Therefore, this study evaluated the presence of this structural disruption of the inner retina in early stages of diabetic neuroretinal disease and its effect on multiple aspects of visual function. Previous studies have described the presence of DRIL in cases of severe NPDR, PDR, current and resolved DME,12,15–17 retinal vein occlusion,14 uveitic cystoid macular edema,15 closed globe trauma,16 retinitis pigmentosa,17 and acute retinal necrosis.27 This study evaluated DRIL in diabetes before the appearance of vascular changes and with DR, before DME and PDR. This study is the first to compare functional performance in subjects with and without DRIL using an automated contrast sensitivity method and three visual field testing strategies, in addition to ETDRS visual acuity testing. Additionally, this study evaluated retinal thickness in subjects with DRIL compared to subjects without DRIL.

Visual function depends on intact organization of the cellular pathways in the retina. All tests of retinal function used in this study have previously revealed neuroretinal impairment in diabetic patients with early DR.2 In this study, ETDRS visual acuity, contrast sensitivity tested using the Quick Contrast Sensitivity method, and SAP detected significant functional differences among controls, diabetic subjects without DRIL, and diabetic subjects with DRIL. SAP was particularly useful in detecting subtle retinal dysfunction in diabetic subjects with DRIL compared to diabetic subjects without DRIL. The significant difference was detected in PSD, but not in MD or FT, suggesting that DRIL may be associated with increased variability across the visual field. Since ETDRS visual acuity, contrast sensitivity, and SAP are nonsellf tests of retinal function, it appears that the dysfunction associated with DRIL is also nonspecific. Testing with FDP and SWAP, selective tests of inner retinal function,22,23 did not detect significant differences among groups; however, future studies with a larger sample size may be able to detect differences.

The development of DRIL in multiple retinal diseases suggests it is not specific to DR but a common response to retinal stress. Recent studies have revealed the cellular basis of retinal lamination during normal fetal development, resulting in distinct layers of cell bodies and synapses.28 The OCT findings of DRIL include loss of definition of the boundaries of the layers,12 consistent with prior reports of loss of inner retinal neurons and thinning of the plexiform layers, particu-

<table>
<thead>
<tr>
<th>Table 3. Comparison of Retinal Function With DRIL.</th>
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<tr>
<td>ETDRS logMAR, (Snellen equivalent)</td>
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<tr>
<td>AULCSF (SD)</td>
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<td>SWAP 24-2</td>
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<tr>
<td>MD (SD)</td>
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<td>PSD (SD)</td>
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<td>FT (SD)</td>
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<td>SAP 24-2</td>
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<td>MD (SD)</td>
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<td>FT (SD)</td>
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<td>FDP 24-2</td>
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<td>MD (SD)</td>
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<tr>
<td>PSD (SD)</td>
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<tr>
<td>FT (SD)</td>
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</table>

* Statistically significant result.

The current study found an association between higher BMI and longer diabetes duration in persons with DRIL, and a greater likelihood of DRIL in subjects with mild to moderate NPDR than in persons without visible DR. However, additional work is needed to better understand the factors that lead to or prevent the presence of DRIL.

The limitations of this study include its cross-sectional nature and modest sample size. Analysis of a larger sample size with similar distribution of males and females in the control group and retinopathy groups will be important to rule out any influence that sex has on retinal structure and function. Furthermore, higher density SD-OCT volume scans may provide better sensitivity for detection of DRIL. Thus, DRIL may be detected in more patients at risk of neuroretinal impairment. The extent of DRIL identified in our patients is difficult to compare to the extent of DRIL described in previous studies because we examined a larger area (6 mm ring of the ETDRS grid centered at the fovea) versus other studies that used the 1 mm ring12,13 and the 3 mm ring.15 This difference needs to be considered when designing future protocols for DRIL in early DR, where it may not be as prevalent as in DME and PDR. The use of 6 mm SD-OCT scans likely contributed to the detection of DRIL in eyes with no or minimal vascular retinopathy. With more advanced technology, it may also be possible to evaluate for DRIL outside the macular area, which may provide further insight into the pathogenesis of diabetic neurodegeneration. Finally, additional longitudinal analysis is necessary to determine whether changes in DRIL predict changes in multiple aspects of visual function long-term.

The findings of this study further emphasize a correlation between neuroretinal structure and function. DRIL could potentially serve as a reliable and readily available tool for screening and monitoring neuroretinal impairment in diabetes.
CONCLUSIONS

This pilot analysis strengthens the potential utility of DRIL as a clinical marker of visual function to test potential novel therapies for diabetic patients with early stages of neuroretinal impairment. Future studies should include a larger cohort and a longitudinal analysis to determine whether DRIL will be useful tool in the detection, monitoring, and management of neuroretinal impairment in DR.

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