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Title

Characterization of the Structural and Functional Alteration in Eyes with Diabetic Macular Ischemia

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Running Head:
DRIL and EZ loss in DMI

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Abbreviations and Acronyms:
AI = acircularity index; **BCVA** = best-corrected visual acuity; **CI** = confidence interval; **DCP =** deep capillary plexus; **DME** = diabetic macular edema; **DMI** = diabetic macular ischemia; **DR** = diabetic retinopathy; **DRIL** = disorganization of the retinal inner layers; **DVC** = deep vascular complex; **DVD** = deep vessel density; **ETDRS** = Early Treatment Diabetic Retinopaty Study; **EZ** = ellipsoid zone; **FAZ** = foveal avascular zone; **FD-300** = parafoveal 300-µm ring vessel density; **GCL** = ganglion cell layer; **GEE** = generalized estimating equation; **ILM** = internal limiting membrane; **INL** = inner nuclear layer; **IPL** = inner plexiform layer; **LLVA** = low-luminance visual acuity; **OCT** = optical coherence tomography**; OCTA** = optical coherence tomography angiography; **OPL** = outer plexiform layer; **OR** = odds ratio; **PDR** = proliferative diabetic retinopathy; **SCP** = superficial capillary plexus; **SD** = standard deviation; **SVC** = superficial vascular complex; **SVD** = superficial vessel density; **VA** = visual acuity; **VD** = vessel density.

Keywords
diabetic macular ischemia; disorganization of the retinal inner layers; ellipsoid zone loss; optical coherence tomography; optical coherence tomography angiography

Abstract

Objective

To investigate the relative effect of disorganization of the retinal inner layers (DRIL) and ellipsoid zone (EZ) loss on visual function in diabetic macular ischemia (DMI).

Design

Prospective cross-sectional observational study.

Participants

Patients with stable treated proliferative diabetic retinopathy (PDR) without center-involved diabetic macular edema were recruited at the Moorfields Eye Hospital from December 2019 to November 2021. The main inclusion criteria were best-corrected visual acuity (BCVA) of at least 40 Early Treatment Diabetic Retinopathy Study letters (Snellen equivalent 20/160) with optical coherence tomography angiography (OCTA) evidence of DMI in at least one eye.

Methods

Each eligible eye of the recruited patients was assessed for BCVA, optical coherence tomography (OCT) and OCTA metrics. The pre-specified OCT parameters were DRIL and subfoveal EZ loss. Generalized estimating equations were used.

Main Outcomes and Measures

The frequency of DRIL and EZ loss, their relative contributions to vision loss, and their associations with microvascular alterations were evaluated.

Results

There were 125 PDR eyes of 86 patients enrolled. A total of 104 (83%) eyes had BCVA ≥70 letters. DRIL was more prevalent than EZ loss (46% [58 eyes] vs. 19% [24 eyes]). On average, the presence of DRIL had a more pronounced impact on vision, retinal thickness, and microvascular parameters than EZ loss. After multivariable adjustment, the odds of co-existing DRIL increased by 12% with every letter decrease in BCVA; however, there was no statistically significant association of subfoveal EZ loss with BCVA. In eyes with DRIL in the absence of EZ loss, the BCVA declined significantly by 6.67 letters compared to eyes with no DRIL nor EZ loss (95% confidence interval [CI], −9.92 – −3.41; $P < 0.001$). However, if DRIL and EZ loss co-existed, the resultant BCVA was 13.22 letters less than eyes without these structural abnormalities (95% CI, −18.85 – −7.59; $P < 0.001$).

Conclusions

In DMI patients with Snellen visual acuity of 20/160 or better, eyes with DRIL are associated with more visual function loss and retinal blood circulation alterations than those with subfoveal EZ loss only.

Manuscript

Visual impairment due to diabetic retinopathy (DR) is a growing public health burden.[1] The most common cause of moderate visual impairment in DR is diabetic macular edema (DME). However, diabetic macular ischemia (DMI) also contributes to visual acuity (VA) loss but is less well studied. VA in eyes with angiographic evidence of DMI may range from normal to severe visual loss. The potential for visual improvement is limited in eyes with severe visual loss (Snellen 20/200 or worse) due to irreversible macular pathology. Therefore, the effectiveness of new therapies in macular diseases is mainly evaluated in eyes with VA better than Snellen 20/200.

The prevalence of DMI increases with worsening severity of DR, with the highest frequency observed in proliferative diabetic retinopathy (PDR). Approximately 77% of the eyes with PDR have angiographic evidence of DMI as defined by the Early Treatment of Diabetic Retinopathy Study (ETDRS) criteria.[2,3] Therefore, it is more feasible to study visual function changes in DMI in eyes with PDR. Eyes with PDR also have the greatest VA variability compared to other severity levels of DR.[4-6] This heterogeneity may be partly explained by the presence of DMI.

With the advent of optical coherence tomography angiography (OCTA), it is now possible to better quantify the microvascular changes in DMI and relate them to changes in visual function. However, these vascular changes may not necessarily cause visual loss unless associated with neuronal derangement. These neuronal changes may manifest as disorganization of the retinal inner layers (DRIL) and ellipsoid zone (EZ) loss. Several reports have shown that these neuronal changes adversely affect visual prognosis.[7,8] However, their relative contribution to VA loss in PDR eyes with DMI is unclear.

Both the presence of DRIL and the length of DRIL have been associated with poor visual performance.[9-12] In addition, studies on DRIL have shown its association with

decreased superficial vessel density (SVD) and deep vessel density (DVD).[13,14] Furthermore, an enlarged foveal avascular zone (FAZ) area is also inclined to have co-existing DRIL.[15] However, it is worth knowing that not all eyes with reduced SVD have DRIL.[16]

Neuronal changes may also occur in the outer retina in DMI, especially in eyes with concomitant or previous DME. In retinal diseases, the loss of EZ in the central one millimeter (mm) subfield on optical coherence tomography (OCT) is a well-established poor visual prognostic indicator.[11,12,17,18] Both the existence and extent of EZ loss are independent factors of VA loss in DR.[11,12] Most importantly, investigators have demonstrated that EZ disruption is more likely to have diminished DVD.[14,19] This finding corresponds to the fact that the retinal circulation provides 10–15% of the oxygen supply for the photoreceptor layer.[20] Together, the combination of DRIL and EZ loss may represent a more severe phenotype of DMI with the worst visual outcome.[21] However, this has not been systematically evaluated. Moreover, the prevalence of DRIL and EZ loss in PDR eyes with DMI with recoverable VA has yet to be determined.

As microvasculature changes in DMI usually extend beyond the fovea, best-corrected visual acuity (BCVA) may not be an ideal visual function test as it is a foveal function. Low luminance visual acuity (LLVA) may provide more information on visual function loss in DMI because it reflects the function of the post-receptoral pathway in the parafoveal area.[22]

Based on these observations, this study aimed to interrogate (1) the prevalence of DRIL and EZ loss and their linear association with BCVA and LLVA in PDR with Snellen acuity better than 20/200; (2) the functional and microvascular parameters associated with DRIL and EZ loss; and (3) the relative contribution of DRIL and EZ loss to VA loss in these eyes.

Methods

This prospective cross-sectional observational study was conducted at the Moorfields Eye Hospital from December 2019 to November 2021. The study adhered to the tenets of the Declaration of Helsinki and was approved by the United Kingdom National Research Ethics Committee Service (19/NI/0030). Informed consent was obtained from all eligible individuals after good explanations.

The electronic medical records from January 2019 to December 2019 were reviewed for patients with stable PDR under follow-up after treatment with panretinal photocoagulation (PRP), with the last session done at least six months previously. All identified potentially eligible patients were invited, and those who attended the screening visit were evaluated for eligibility.

Patients were included if they had at least one eye with: (1) stable treated PDR, defined as no active neovascularization in the past six months following PRP; (2) an enlarged and irregular FAZ of $\geq$0.5 mm^2 in the superficial vascular complex (SVC) or parafoveal capillary dropout in one quadrant or more if the FAZ area was less than 0.5 mm^2 on OCTA; and (3) BCVA of at least 40 ETDRS letters (Snellen equivalent 20/160). Both eyes were enrolled if eligible.

The key exclusion criteria were active PDR, defined as neovascularization of the disc (NVD), neovascularization elsewhere (NVE), and vitreous hemorrhage; the presence of OCT evidence of center-involved DME; history of intravitreal injection (IVI) in the past six months; and any condition that, in the investigator's view, could affect VA, such as visually disabling cataract, epiretinal membrane, or other macular co-morbidities.

Visual Acuity Assessment

A masked optometrist assessed the patients' refraction status, BCVA, and LLVA in a standardized clinic. In brief, the BCVA was obtained using a retro-illuminated high-contrast ETDRS chart (180 candela/m^2, Precision Vision, Bloomington, IL, USA) at four meters

when the room lights were dimmed. The LLVA was examined after placing a neutral density filter in front of the ETDRS chart without changing the light settings, reducing the luminance by 2.0 log units.[23] Low-luminance deficiency (LLD) was defined as the letter score of BCVA minus LLVA.

OCT Examination

All patients underwent macular scans centered at the fovea on Spectralis HRA-OCT (Heidelberg Engineering, Germany). The OCT volume scan was performed on a 20 x 20-degree cube with 49 raster lines, each containing 1064 pixels, scanned at an interval of 120 µm apart with a speed of 40000 scans/second.[24,25] Only images with a signal strength of ≥20 dB were used for analysis.

A 1,3,6-mm ETDRS grid overlay was placed centered at the foveal depression. The central subfield thickness (CST) was defined as the average retinal thickness over the central 1-mm diameter circle at the fovea, automatically measured by the inbuilt software. DRIL and EZ loss were assessed across the seven 1-mm foveal B-scans, including three scans above and below the central line. Within this 1 x 0.72 mm area, DRIL was judged by the inability to distinguish the ganglion cell-inner plexiform layer (GCL/IPL) complex, inner nuclear layer (INL), and outer plexiform layer (OPL) contours,[9,10] and its presence was objectively defined as the sum of ambiguous boundaries of ≥3500 µm. The EZ loss in the same area was described as a definite break in the EZ after excluding other causes of EZ disruption, such as a previous macular laser scar. A sum of >0 µm EZ loss was called EZ loss present. Two examiners (W.S.T. and S.T.) masked to the associated VA measured the total length of DRIL and EZ loss. Any discrepancies found were arbitrated by a senior third grader (S.S.).

OCTA Acquisition

The device used for acquiring OCTA scans was the Optovue imaging system (Avanti RTVUE-XR; Fremont, CA, USA, version 2018.0.0.18). It is a spectral-domain OCTA with a

light source of 840 nm wavelength. The inbuilt split-spectrum amplitude-decorrelation angiography (SSADA) technology can produce denoised high-quality scans at a 70000 A-scans/second speed.[26,27] The device is also equipped with projection artifacts removal and motion artifects correction algorithms.[28,29] The software did the FAZ delineation; however, manual adjustments were made if vessels or suspended scattering particles in motion (SSPiM) were erroneously included.[30] Segmentation errors, if any, were also manually rectified.

The retinal layers were segmented automatically into two major components by the inbuilt software- the superficial vascular complex (SVC) and the deep vascular complex (DVC). The SVC, defined as the slab between the internal limiting membrane (ILM) and 9 μm above the IPL, contains both the nerve fiber layer vascular plexus and the superficial capillary plexus (SCP). The DVC, starting from 9 μm above the IPL to 9 μm below the OPL, comprises the intermediate capillary plexus (ICP) and the deep capillary plexus (DCP).

Only those images focused on the 3 x 3 mm fovea with a quality score of ≥5 were included for OCTA analysis. The readings of the FAZ-associated parameters were collected, including the FAZ area and the FAZ perimeter. The FAZ acircularity index (AI) was quantified using the formula defined as the ratio of the measured perimeter to the circumference of a perfect circle with the same area as the studied FAZ.[31] In addition, the parafoveal 300-μm ring vessel density (FD-300), indicating the capillary vessel density (VD) extended 300 μm beyond the FAZ border, was recorded. We also obtained the microvascular indexes, including SVC VD (SVD) and DVC VD (DVD), from the whole image and parafoveal region (the ring area between the 1- and 3-mm diameter circle).

Statistical Analysis

Demographic, clinical and ocular characteristics were summarized in the whole cohort. As some subjects were eligible in both eyes, generalized estimating equations (GEE) were used

to account for inter-eye correlation, assuming an unstructured correlation structure and robust standard errors. Univariate and demographic-adjusted analyses using GEE models with the logit link function for the binary outcomes of DRIL and EZ loss were used to obtain the odds ratio (OR) of risk factors and their 95% confidence intervals (CI). Age, gender and duration of diabetes were adjusted for DRIL, while only age was adjusted for EZ loss due to the low numbers with EZ loss. Univariate and adjusted analyses using GEE models were also used to obtain the mean difference with 95% CI of risk factors and the interaction effect between the presence of DRIL and EZ loss for the continuous outcome BCVA. In addition to a priori confounding demographic variables (age, gender and duration of diabetes), history of vitrectomy was also adjusted for the outcome of BCVA as it reached statistical significance in the univariate analysis. Cohen's Kappa was employed to test the intrarater and interrater reliability.[32] Statistical analysis was performed using the Stata MP version 15.[33] Two-sided *P* values of 0.05 or lower were considered statistically significant.

Results

A total of 209 patients with a history of PRP were identified. Among them, 115 patients attended the screening visit, while the rest refused (n = 57) or did not respond (n = 37). At screening, 29 patients failed the eligibility criteria for the following reasons: Poor VA <40 ETDRS letters (n = 2), DR severity less than PDR (n = 5), active PDR (n = 4), center-involved DME (n = 1), not fulfilling the OCTA definition of DMI (n = 7), IVI within recent six months (n = 2), visually significant cataract (n = 2), severe epiretinal membrane (n = 1), macular scars (n = 1), tractional retinal detachment (n = 1), neovascular glaucoma (n = 2), and inability to cooperate with the examinations (n = 1).

Overall, the study recruited 125 eyes of 86 stable treated PDR patients, and 47% were eligible bilaterally. Fourteen eyes were corrected for FAZ delineation errors, and eight were

excluded from OCTA analysis as the quality score was less than five. The average age was 56.6 ± 12.6 years. Baseline demographics and ocular characteristics are summarized in **Table 1**. These eyes had a mean BCVA of 77 ± 9 ETDRS letter score, and the majority (83%) presented with a BCVA of ≥70 letter score. Their average LLVA was 66 ± 12 letter score.

The average FAZ area was 0.55 ± 0.39 mm^2 with a mean FAZ perimeter of 3.33 ± 1.39 mm. The calculated AI was 1.31 ± 0.20. The total SVD and DVD in the 3 x 3 mm area were 36.49 ± 5.30% and 41.77 ± 4.86%, respectively. The parafoveal VD highly correlated with the 3 x 3 mm whole image VD, showing 38.35 ± 5.96% and 43.39 ± 5.01% in the SVC and the DVC, respectively. The average FD-300 was 42.80 ± 5.01%.

Regarding OCT examinations, the average CST was 263 ± 42 μm. Approximately half the eyes had DRIL (46%), and the mean DRIL length was 3365 ± 1949 μm. However, only one-fifth (19%) exhibited EZ loss with a median of 158.5 μm (interquartile range from 92.5 to 661.5 μm). The intrarater agreement was 100% for DRIL and 76% for EZ loss, and the interrater agreement was 73% for DRIL and 92% for EZ loss. The results suggested good to strong intrarater and interrater reliability.

The vascular and functional metrics associated with DRIL and subfoveal EZ loss are summarized in **Tables 2 & 3**. Generally, the presence of DRIL exhibited a more pronounced influence on all the functional and vascular metrics than the presence of subfoveal EZ loss in DMI (**Figures 1 and 2**).

The univariate analysis showed that a longer duration of diabetes, poorer visual acuity, larger FAZ parameters (including area, perimeter, and AI), worse OCTA VD (whole image and parafoveal SVD or DVD), and a thinner CST were associated with DRIL (**Table 2**). The *P* values remained statistically significant even after demographic adjustment except for the duration of diabetes. However, age, gender, LLD, previous macular laser, and history of DME and vitrectomy did not exhibit a definite role in DRIL formation.

On the other hand, no specific risk factors were found to be associated with subfoveal EZ loss in DMI (**Table 3**). Notably, the *P* values for LLD, previous DME, vitrectomy, and macular laser did not reach statistical significance. Contrary to DRIL, the presence or absence of subfoveal EZ loss was not significantly altered by the size of FAZ, the length of the perimeter, the magnitude of AI, the thickness of the central macula, or any VD parameters on OCTA. The only exception was the BCVA— for every one letter decrease in BCVA, the odds for EZ loss increased by 6% (OR, 1.06; 95% CI, 1.01–1.11; $P = 0.03$). However, this significance failed to maintain after age adjustment.

At a closer inspection of those influential factors of DRIL after demographic adjustment (**Table 2**), the odds of having co-existing DRIL increased by 12% with every one letter decrease in BCVA (OR, 1.12; 95% CI, 1.06–1.18; $P < 0.001$). Moreover, the odds of DRIL presence increased by 1.42 times per 0.1 mm^2 enlargement in the FAZ area (OR, 1.42; 95% CI, 1.02–1.99; $P = 0.04$). For example, an eye with a FAZ area of 0.6 mm^2 would be 2.84 times the odds of having DRIL than an eye with a FAZ area of 0.4 mm^2. Furthermore, the multivariable analysis showed that the possibility of DRIL presence increased most in the decline of the whole image DVD among all VD parameters per every 1%-point decrease (OR, 1.28; 95% CI, 1.15–1.44; $P < 0.001$). Of note, the risk of DRIL was higher with every 1%- point decline in the DVD than in the SVD.

Table 4 illustrates the impact of structural derangement in DMI on different parameters in more detail by combining the presence (+) or absence (–) of DRIL and EZ loss. Overall, the presence of DRIL alone was associated with more functional loss (BCVA, LLVA) and worse OCTA metrics (FAZ area, perimeter, AI, whole image and parafoveal SVD or DVD, FD-300) than EZ loss alone. The loss of EZ was not significantly associated with the worsening of any OCTA metrics except the FAZ perimeter and AI. However, concurrent

DRIL (+) and EZ loss (+) resulted in the worst performance in almost every item, except the FAZ area and perimeter.

On further examining the relative contribution of DRIL and EZ to visual function after adjusting for demographic variables and history of vitrectomy (**Table 5**), we discovered that the presence of DRIL was associated with a decrease in BCVA by 7.68 letters compared to no DRIL presence (95% CI, −10.55 – −4.82; $P < 0.001$) regardless of EZ loss. The influence of EZ loss was neither remarkable in the unadjusted nor adjusted analysis compared to no EZ loss. In the presence of DRIL without EZ loss, the BCVA declined by 6.67 letters compared to eyes with no DRIL and no EZ loss (95% CI, −9.92 – −3.41; $P < 0.001$). If DRIL presented in addition to EZ loss, the BCVA would be lowered by 13.22 letters compared to eyes without structural abnormalities (95% CI, −18.85– −7.59; $P < 0.001$).

Discussion

The key findings of our study are: Firstly, approximately 80% of the PDR patients with DMI in our study cohort had a relatively good BCVA of 70 letters or more, and about half of them had LLVA of 70 letters or better. Secondly, in these eyes with DMI, DRIL was more prevalent than EZ loss (46% versus 19%, respectively). Thirdly, DRIL alone was associated with worse functional and microvascular metrics than those eyes with EZ loss only. Fourth, concurrent DRIL and EZ loss was present in only 10% of the eyes; however, these eyes had the worst functional outcomes. Finally, although on average, both the DVD and SVD were decreased in DRIL, the decrease in DVD was more prevalent.

Most patients with OCTA evidence of DMI in this cohort had good visual acuity. Approximately 40% of the eyes with BCVA of 70 letters or more had DRIL. Therefore, the presence of DRIL may not be a surrogate for severe visual impairment. Considering that DRIL represents the derangement of the post-photoreceptoral pathway, visual acuity may

only be affected when the derangement reaches a certain threshold.[9,34] This threshold may depend both on the duration and quantity of DRIL.

In our study, we did not average the DRIL length across the scans but used the total length of DRIL in each of the seven 1-mm foveal B-scans to obtain the true extent of DRIL. However, numerous studies have scrutinized the relation between DRIL and VA using various definitions, and all have observed that, on average, DRIL is associated with VA loss. For example, DaCosta et al. used multivariable regression analysis in a cross-sectional study to demonstrate that the horizontal DRIL length was significantly associated with VA (P = 0.01).[11] Endo et al. further illustrated that the length of DRIL was positively correlated with the logarithm of the minimum angle of resolution (logMAR) VA.[12] Moreover, DRIL was proposed as a surrogate predictor of future VA in a longitudinal study by Sun et al., who reported that an extent of 300 µm DRIL at four months was predictive of 1-line VA loss at eight months in center-involved DME.[9] The negative influence of DRIL on VA held true in DME-resolved eyes.[10] We excluded eyes with concomitant DME, but some of these eyes may have had previous DME. Our study showed that eyes with DRIL had approximately 7.68 letters worse BCVA than non-DRIL eyes regardless of EZ condition. The efficacy of novel therapeutic agents in preventing visual loss in DMI may be better evaluated in eyes without DRIL or DRIL with good VA.

We also evaluated the relation between the OCTA metrics and DRIL. Importantly, we observed that an enlarged FAZ area was associated with DRIL. This finding substantiates observations made in other studies.[15,35] Apart from comparing the average size of the FAZ area between eyes with and without DRIL, our study specifically pointed out that the odds of co-existing DRIL increased by 42% with every 0.1 mm^2 increase in the FAZ area (95% CI, 1.02–1.99; P = 0.04) after demographic adjustment. However, the cross-sectional research

design in all these studies limits inferences about the natural history of DRIL development and progression.

When considering OCTA metrics of VD in DMI, the key observations were a more profound association of decreased DVD than SVD in eyes with DRIL and a close relationship between DRIL and AI of 1.5 or more. The relation between DRIL and VD in different layers is still under debate. Whereas some researchers support that DRIL is predominantly associated with SVC deficiency,[34,35] others believe that DRIL is also associated with DVC ischemia besides SVC insufficiency.[14] Our study reinforced the latter findings by comparing the odds ratios between the two vascular parameters. The multivariable analysis showed that every 1%-point decrease in SVD increased the chance of DRIL presence (OR, 1.13; 95% CI, 1.04–1.23; $P = 0.006$), but the same amount of decline in DVD appeared to have a stronger association with DRIL (OR, 1.28; 95% CI, 1.15–1.44; $P < 0.001$). However, the discrepancy may also reflect different DMI phenotypes.

Our study did not find a significant role of subfoveal EZ loss alone in visual performance in DMI with VA better than Snellen 20/200. Sun et al. also reported similar findings despite a wider visual acuity range (logMAR 0.28 ± 0.25). After multivariate modeling, they reported that the positive correlation between EZ breaks and baseline logMAR VA became insignificant.[9] We attributed the insignificance in the present study to the small number of eyes having subfoveal EZ disruption (24 eyes). As countless multivariate regression analysis studies have demonstrated that EZ loss length contributes to visual impairment significantly and proportionally,[11,12,17,18] we are convinced that photoreceptor injury should result in vision loss. However, given the low prevalence of EZ loss in our study, we inferred that EZ loss might be a late event in DMI,[34] as we showed that eyes with concurrent DRIL and EZ loss have the worst visual outcome. It may also represent a group

with previous DME. However, we could not deduce this from this cohort as only 14% of our study participants had previous DME.

We also showed a synergistic adverse effect of DRIL and EZ loss on vision. The finding also points to the contribution of DCP changes in DMI as DCP aids in maintaining photoreceptors. In 2016, Scarinci et al. first described eight DMI eyes with topographic relation between flow abnormalities in the DCP and attenuated signals in the outer retinal layers.[36] Following the first publication, Nesper et al. reported quantitative results that a reduced cone heterogeneity packing index was associated with parafoveal DCP non-perfusion in ten eyes.[8] In a multivariate linear regression model analyzing sixty-seven resolved DME, Moon et al. discovered that the recovery of EZ integrity significantly relied on the baseline DVD.[37] Our study indicated that the DVD loss was not significantly different between eyes with or without EZ loss. Although it is widely accepted that the DVC contributes 15% of the oxygen supply to the photoreceptor layer,[38] our study showed that DVD loss was seen more in DRIL rather than subfoveal EZ loss in DMI. One of the possible explanations could be that the function of the outer retina is more dependent on the choroid.[34,38]

There are several strengths of this study. To the best of our knowledge, this is the first observational study that prospectively recruited eyes with DMI in stable treated PDR and included only eyes with VA better than Snellen 20/200. We also applied stringent OCTA criteria to define DMI. Moreover, we measured DRIL and EZ loss length across seven scans within the central 0.72 x 1 mm square zone rather than one cross-sectional scan at the center, enabling the quantitative data from anatomical structure to reflect the true foveal function more reliably. We further employed GEE and multivariable statistical models to estimate the real effect of isolated DRIL and EZ disruption on vision and central microvascular

parameters. We believe our findings will offer researchers valuable baseline references for future studies investigating the natural history of DMI.

We also acknowledge some limitations of this study. First, the cross-sectional nature of this study design inhibited us from predicting the prognosis of DMI. Second, measuring DRIL and EZ loss length on seven OCT scans can be time-consuming and subject to detection bias. Third, the conclusions drawn from these pre-selected patients may preclude the application to non-PDR eyes, although the prevalence of DMI is lower in these eyes.

In summary, our study demonstrates that DRIL correlates better with visual acuity loss than subfoveal EZ disruption in DMI patients with VA better than Snellen 20/200. Many patients with DRIL still have good visual acuity. In addition, DVD deficiency is more frequently found than decreased SVD in eyes with DRIL. Finally, concurrent DRIL and EZ loss is associated with the poorest microvascular supply, and this synergy results in the worst visual performance.

Figure Legends

Figure 1. The proportion of disorganization of the retinal inner layers (DRIL) in different characteristics. DRIL was more likely to present in eyes with decreased vision, an enlarged FAZ area, an elongated FAZ perimeter, a larger AI, a thinner CST, and a worse whole image SVD and DVD in diabetic macular ischemia.

Abbreviations: AI = acircularity index; BCVA = best-corrected visual acuity; CST = central subfield thickness; DRIL = disorganization of the retinal inner layers; DVD = deep vessel density; FAZ = foveal avascular zone; FD-300 = parafoveal 300-μm ring vessel density; LLVA = low-luminance visual acuity; SVD = superficial vessel density.

Figure 2. The proportion of ellipsoid zone (EZ) loss in different characteristics. EZ loss in diabetic macular ischemic eyes was only associated with a lower best-corrected visual acuity (BCVA). However, after multivariate adjustment, the association lost its significance (Table 3). The foveal avascular zone and retinal microvascular parameters were generally unaffected, contrary to the extensive alteration presented by disorganization of the retinal inner layers (DRIL).

Abbreviations: AI = acircularity index; BCVA = best-corrected visual acuity; CST = central subfield thickness; DRIL = disorganization of the retinal inner layers; DVD = deep vessel density; EZ = ellipsoid zone; FAZ = foveal avascular zone; FD-300 = parafoveal 300-μm ring vessel density; LLVA = low-luminance visual acuity; SVD = superficial vessel density.

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