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Disorganization of retinal inner layers correlates with ellipsoid zone disruption and retinal nerve fiber layer thinning in diabetic retinopathy

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ABSTRACT

Purpose: To study the correlation between disorganization of inner retinal layer (DRIL) and macular thickness parameters, ellipsoid zone (EZ) disruption and retinal nerve fiber layer (RNFL) thickness on spectral domain optical coherence tomography (SD-OCT) in diabetic retinopathy (DR), for the first time.
Methods: A tertiary care center-based cross-sectional study was undertaken. One hundred and four consecutive study subjects of type 2 diabetes mellitus were included: diabetes mellitus with no retinopathy (No DR) ($n = 26$); non-proliferative DR (NPDR) ($n = 26$); proliferative DR (PDR) ($n = 26$) and healthy controls ($n = 26$). Best Corrected Visual Acuity (BCVA) was measured on the logarithm of the minimum angle of resolution (logMAR) scale. Clinician-friendly, SD-OCT based, grading systems were created for DRIL and EZ disruption, within the macular cube. DRIL was graded as: grade 0, DRIL absent; and grade 1, DRIL present. EZ disruption was graded as; Grade 0: Intact EZ; Grade 1: Focal disruption and Grade 2: Global disruption. Every study subject underwent RNFL thickness analysis.
Results: DRIL was significantly associated with increase in severity of DR.Pearson correlation analysis showed significant positive correlation between DRIL and CST CAT and grades of EZ disruption . However, a significant negative correlation was found between DRIL and RNFL thickness .
Conclusion: Presence of DRIL correlates with severity of DR, EZ disruption and RNFL thinning.

1. Introduction

Annual incidence and progression of diabetic retinopathy (DR) ranges from 2·2% to 12·7% and 3·4% to 12·3% respectively.[1] Diabetic retinopathy (DR) is among the most common diabetic complications and ranks as the fifth most common cause of global vision loss.[2] Recent analysis suggested that the number of people with diabetic retinopathy would increase to 191 million by 2030.[3]

Optical coherence tomography (OCT) is a powerful, non-invasive, transpupillary imaging modality that allows in vivo high-resolution cross-sectional analysis of the retinal microanatomy.[4]

Disorganization of the inner retinal layers (DRIL) is defined as the failure to discriminate the inner retinal layer boundaries; any of the boundaries of ganglion cell layer-inner plexiform (GCL-IPL) complex, and inner nuclear layer (INL) and outer plexiform layer (OPL). DRIL in the 1-mm foveal area has a detrimental effect on visual acuity (VA), and is predictive of VA in eyes with center-involved diabetic macular edema (DME). Early change in DRIL prospectively recognizes eyes with high probability of subsequent improvement or decline in VA.[5]

Ellipsoid zone (EZ), traditionally ascribed to as inner/outer segment (IS/OS) junction[6] of the photoreceptors is the second hyper-reflective band, which corresponds with the anatomical location of the ellipsoid fraction of the photoreceptors inner segment.[7]

The extent of the EZ disruption is a predictor of visual outcome in diabetic macular edema. The integrity and intensity of the EZ has been

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shown to be associated with the visual function and is an important indicator of visual outcomes in many retinal conditions.[8,9]

The retinal nerve fiber layer (RNFL) is comprised of axons derived from ganglion cells. Earlier studies have demonstrated RNFL thinning or defects in patients with diabetes mellitus.[10–15]

The present study was undertaken to assess the correlation of DRIL with macular thickness parameters, EZ disruption and RNFL thickness, for the first time.

2. Methods

The present study was performed in accordance to the tenets of the declaration of Helsinki. Study was undertaken after institutional review board clearance and a written informed voluntary consent. The study was a tertiary care center-based cross sectional study. Diagnosis of type 2 diabetes mellitus was made according to the American Diabetes Association (ADA) guidelines which include fasting plasma glucose level ≥126 mg/dl and two hour plasma glucose level ≥200 mg/dl during an oral glucose tolerance test.[16] Sample size was calculated to be 104. The sample size was calculated using 95% confidence limits with the 2-sided α level of 0.05, the power of the study 80% with respective z-value of 0.84.

Seventy-eight consecutive cases of type 2 diabetes mellitus, in the age group of 40 to 70 years were included. Cases were divided into three groups based on the early treatment diabetic retinopathy study (ETDRS) classification[17]: diabetes patients without retinopathy (NO DR, n = 26), non-proliferative diabetic retinopathy (NPDR, n = 26), and proliferative diabetic retinopathy (PDR, n = 26). Twenty-six healthy controls attending ophthalmology outpatient department for refraction, between age 40 years and 70 years, were also included. Patients with any ocular or systemic disease affecting retinal vascular pathology (hypertension), glaucoma, age-related macular degeneration, intraocular inflammation, cases with previous intravitreal injection(s), surgical or laser intervention were excluded. Study participants with media haze at any level giving signal strength of <7, on spectral-domain optical coherence tomography (SD-OCT) and on medications, vitamin, or antioxidant supplements were excluded. Cases with systemic diseases like end stage renal disease, nervous disease (Alzheimer's disease, peripheral neuropathy), cardiovascular disease, tuberculosis, chronic liver disease, cancer were also excluded.

Age and gender was documented. The best-corrected visual acuity was documented on the logarithm of the minimum angle of resolution (logMAR) scale. All study participants underwent detailed fundus evaluation using stereoscopic slit lamp biomicroscopy and indirect ophthalmoscopy. Digital fundus photography and fluorescein angiography were done using Zeiss fundus camera FF 450 Plus (Carl Zeiss Meditec, Jena, Germany). All the cases and controls underwent SD-OCT using macular cube 512 × 128 feature of Cirrus High Definition SD-OCT (Carl Zeiss Meditec Inc., CA). Central subfield thickness (CST) and cube average thickness (CAT) were documented. CST was defined as the retinal thickness of the central 1-mm-diameter circle of the ETDRS grid. CAT was defined as an overall average thickness for the internal limiting membrane-retinal pigment epithelium tissue layer over the entire 6 × 6 mm square scanned area. RNFL thickness analysis was performed using the optic disc cube 200 × 200 feature. The average RNFL thickness was documented. Clinician-friendly, SD-OCT based, grading systems were created, by the authors, for DRIL and EZ. Disorganization of inner retinal layers was defined as the inability to delineate the inner retinal layer boundaries; any of the boundaries of ganglion cell layer-inner plexiform (GCL-IPL) complex, INL and OPL. DRIL was graded as: grade 0, DRIL absent; and grade 1, DRIL present. DRIL was considered to be absent, if intraretinal cysts were observed in outer nuclear layer, resulting in overall retinal thickening, but the inner retinal layers could still be demarcated. Ellipsoid zone was defined as the second hyper reflective band, of the outer retinal layers, on horizontal and vertical SD-OCT scans. Ellipsoid zone disruption was graded as; Grade 0: Intact photoreceptor EZ; Grade 1: Focal disruption (subfoveal localized involvement) and Grade 2: Global disruption (generalized involvement within the macular cube).[18] To ensure validity signal strength of 7 and three same consecutive readings of the numerical data (CST, CAT) were recorded. Two experienced observers masked to the status of diabetic retinopathy assessed the grades of DRIL and EZ disruption. The intergrader agreement was assessed using Cohen's Kappa.[19] Blood samples were collected and fasting and post-prandial blood glucose levels were estimated using the glucose oxidase method. Glycated hemoglobin was measured on autoanalyser using standard protocol.

2.1. Statistics

Data were summarized as Mean ± SE (standard error of the mean). The intergrader agreement was assessed using Cohen's Kappa. Continuous two independent groups were compared by Student's *t*-test. Continuous >2 independent groups were compared by one-way analysis of variance (ANOVA) and the significance of mean difference between the groups was done by Newman-Keuls post hoc test after ascertaining normality by Shapiro-Wilk's test and homogeneity of variance between groups by Levene's test. Categorical (discrete) groups were compared by chi-square (χ^2) test. Pearson correlation analysis was done to assess association between the variables. A two-tailed ($\alpha = 2$) $p < 0.05$ was considered statistically significant. Interpretation and analyses of obtained results was performed on statistical package for social sciences (SPSS, Inc., Chicago, IL) software (Windows version 17.0).

3. Results

The demographic, biochemical, clinical, and SD-OCT based data according to severity of DR is shown in Table 1. Mean duration of diabetes mellitus, in years, was 7.23 ± 5.68 in No DR, 9.83 ± 5.43 in NPDR, and 10.89 ± 4.29 in PDR groups respectively. Analysis of variance showed no statistically significant difference in age among the study groups ($F = 0.03$, $p = 0.90$).The chi-square test revealed similar sex proportions among all four groups ($\chi^2 = 4.90$, $p = 0.20$).

The mean glycated hemoglobin in the control, No DR, NPDR, and PDR groups was 5.43 ± 0.11, 7.81 ± 0.50, 8.22 ± 0.42, 8.81 ± 0.51 respectively. The difference in the glycated hemoglobin levels was statistically significant between the study groups ($F/\chi^2 = 12.60$, $p < 0.001$). The intergrader agreement for DRIL and EZ disruption was high with a Cohen's Kappa of 0.85.

The Pearson correlation analysis showed a significant and positive correlation of logMAR BCVA with CST ($r = 0.71$, $p < 0.001$), CAT ($r = 0.72$, $p < 0.001$), DRIL ($r = 0.78$, $p < 0.001$) and EZ disruption ($r = 0.82$, $p < 0.001$). A significant negative correlation was observed between VA and RNFL ($r = -0.39$, $p < 0.001$).

Student's *t*-test showed significant high LogMAR BCVA in cases with DRIL present as compared to DRIL absent (0.28 ± 0.03 vs. 0.99 ± 0.06, $t = 11.61$, $p < 0.001$). Similarly, ANOVA showed significant positive correlation between mean BCVA levels and EZ disruption ($F = 85.6$, $p < 0.001$). Further, Newman-Keuls test showed significant high logMAR BCVA in focal and global disruption as compared to intact EZ ($p < 0.001$) (Figs. 1 and 2).

Pearson correlation analysis showed a significant and positive correlation CST ($r = 0.78$, $p < 0.001$), CAT ($r = 0.76$, $p < 0.001$), EZ grade ($r = 0.94$, $p < 0.001$) with DRIL. However, a significant and negative correlation was observed between DRIL and RNFL ($r = -0.41$, $p < 0.001$).

Table 2 shows significant correlation between Correlation of DRIL with macular thickness parameters, grades of EZ disruption and RNFL thickness.

4. Discussion

DRIL is a novel, noninvasive parameter that appears to be highly correlated with VA in eyes with DR. Early changes in DRIL extent are also

Table 1
Demographic, clinical and optical coherence tomography-based macular thickness parameters, ellipsoid zone disruption and retinal nerve fiber layer thickness (n = 104) (Mean ± SE).

Variables	Controls (n = 26)	NO DR (n = 26)	NPDR (n = 26)	PDR (n = 26)	F/χ^2 value	*p* value
Age (yrs)	53.50 ± 1.61	54.12 ± 1.21	53.81 ± 1.40	55.64 ± 1.72	0.03	0.90
Sex						
Female	10	15	8	11	4.90	0.20
Male	16	11	18	15		
HbA1c (%)	5.43 ± 0.11	7.81 ± 0.50	8.22 ± 0.42	8.81 ± 0.51	12.60	<0.001
BCVA (logMAR)	0.09 ± 0.02	0.36 ± 0.04	0.68 ± 0.07	1.18 ± 0.02	112.61	<0.001
CST (μm)	246.91 ± 2.63	255.55 ± 12.22	330.33 ± 23.81	510.09 ± 23.90	42.40	<0.001
CAT (μm)	256.62 ± 1.08	280.23 ± 8.90	298.51 ± 7.81	390.22 ± 10.52	52.90	<0.001
DRIL						
Absent	26	26	12	3	52.00	<0.001
Present	0	0	14	23		
EZ grade[a]						
Intact EZ	26	26	10	2	67.21	<0.001
Focal disruption	0	0	9	6		
Global disruption	0	0	7	18		
RNFL (μm)	89.41 ± 0.33	82.32 ± 2.91	79.51 ± 3.55	74.00 ± 2.29	6.70	<0.001

[a] Dichotomous grading for EZ shows intact EZ in controls (n = 26) and No DR (n = 26); disrupted EZ in NPDR (n = 16) and PDR (n = 24), F/χ^2 value = 70.20, $p \leq 0.001$.

predictive of longer-term VA outcomes in eyes with DME. The present study highlighted significant correlation of DRIL with severity of DR. Presence of DRIL was observed to be significantly associated with increase in CST, CAT and EZ disruption and decrease in RNFL thickness. In an earlier study, cases with DRIL have been reported to be associated with diminished retinal function compared to those without DRIL. Further, disorganized retinal lamination may be an early cellular consequence of diabetes leading to DRIL and may serve as a reliable anatomical biomarker for decreased retinal function in early DR.[20]

Three integral proteins, occludin,[21] claudins[22] and junctional adhesion molecules (JAMs)[23] form tight junction complexes at the inner and outer blood retinal barrier (BRB) and the external limiting membrane (ELM).[24] These serve as mechanical and physiological barrier thereby, selectively preventing the passage of molecules into the extracellular tissue of the retina.[25] ELM is an intercellular junction between the Muller cells and photoreceptor cells and has barrier properties against macromolecules.[26] The ELM also contributes to the blood-retinal barrier function.[24] Disruption of ELM leads to photoreceptor damage.[27,28]

Chronically elevated blood glucose, high cholesterol, hypoxia, ischemia, accumulation of oxygen free radicals, advanced glycation end (AGE's) products and protein kinase C have been implicated in the pathogenesis of DME.[29,30] Endothelial cells, pericytes, Muller cells, microglia, astrocytes, retinal pigment epithelium and neurons have all been known to produce vascular endothelial growth factor (VEGF).[31] Elevated VEGF levels have been found to correlate with increased vascular permeability, in conjunction to decreased occludin levels in vitreous of diabetic patients.[32] Increase in expression of VEGF results in breakdown of BRB[33,34] and ELM disruption.[27] Starling's law emphasizes that the chief driving force is the pressure gradient between the oncotic and hydrostatic forces of liquid flow which results in macular edema. When local compensatory mechanisms are exhausted, vasogenic edema develops.[29] Breakdown of BRB results in accumulation of plasma proteins, which exert a high oncotic pressure resulting in interstitial edema. Dysfunction of the inner and outer BRB leads to accumulation of sub- and intra-retinal fluid in the inner and outer-plexiform layers, thus contributing to DRIL.

Cystoid spaces extending from the INL to the OPL are accompanied by ELM disruption in DME, signifying disturbance of the Muller cells.[35] Muller cells lose their occludin content at the ELM level, which leads to cyst formation.[26] With increasing severity of retinopathy, ELM disruption also leads to increased grades of EZ disruption and decrease in VA.[27]

Cases with diabetes but no DR and also with mild DR have been demonstrated to be associated with decrease in RNFL thickness, thereby signifying that neurodegeneration occurs prior to retinal vascular changes.[36] RNFL thinning is attributed to diabetic induced alterations in microcirculation viz. leukostasis,[37] vascular obliteration,[38] and degenerative changes of the capillary basal membrane.[39,40] Vasoconstriction, induced by diabetes, diminishes the blood supply from superficial capillaries to the nerve fiber layer and optic nerve head. Metabolic and oxidative stress of diabetes leads to increased sensitivity of ganglion cell, which results in nerve cell loss.[41] With increasing severity of retinopathy, DRIL leads to the destruction and stretching of its bipolar, amacrine, and horizontal cells, thus preventing the relay of information from photoreceptors to ganglion cells,[5] resulting in RNFL thinning with subsequent poor visual acuity.

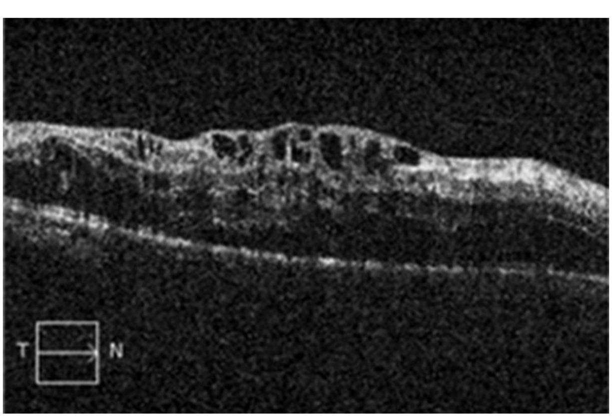

Fig. 1. DRIL present with EZ disruption in DME.

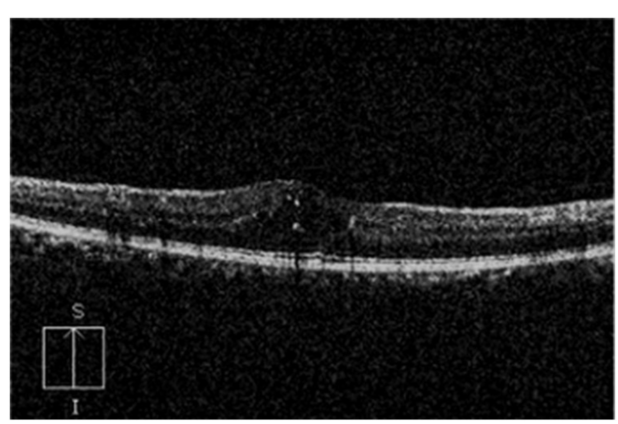

Fig. 2. DRIL absent with intact EZ in DME.

Table 2
Correlation of DRIL with macular thickness parameters, grades of EZ disruption and RNFL thickness (n = 104).

Variable	DRIL		t/χ^2 value	p value
	Absent (n = 67)	Present (n = 37)		
CST (μm)				
Mean ± SE	246.02 ± 2.91	453.42 ± 20.43	11.72	<0.001
CAT (μm)				
Mean ± SE	266.04 ± 2.42	362.05 ± 9.81	10.96	<0.001
EZ grade[a]				
Intact EZ	64	0	84.27	<0.001
Focal disruption	3	12		
Global disruption	0	25		
RNFL (μm)				
Mean ± SE	88.43 ± 1.38	77.51 ± 2.40	4.17	<0.001

[a] Dichotomous grading of EZ shows EZ disruption (n = 37) with presence of DRIL and EZ disruption (n = 3) with absence of DRIL; t/χ^2 value = 91.89, $p \leq 0.001$.

The present study highlighted that DRIL correlated with severity of DR and associated decrease in visual acuity. DRIL also correlated with macular thickness parameters, grades of EZ disruption and RNFL thickness. DRIL thereby may be useful marker allowing better stratification of DR.

The limitation of this study was its cross sectional design. In conclusion, DRIL correlates with EZ disruption and RNFL thinning in diabetic retinopathy.

5. Conclusion

Presence of DRIL correlates with severity of DR, EZ disruption and RNFL thinning in diabetic retinopathy. The results suggest that the association of DRIL with severity of DR is more consistent than that observed with other previously measured imaging markers, making foveal DRIL a potentially highly valuable tool of substantial clinical and investigative importance that could significantly change the approach to ophthalmic counseling and therapeutic management in patients with DR.

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