Assessment of Inner and Outer Retinal Layer Changes in Diabetic Retinopathy

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Abstract: *Spectral domain-Optical coherence tomography (SD-OCT) provides information about the in-vivo histology of the retina. Diabetic retinopathy is associated with structural changes within the retina. Recently, disorganization of the inner retinal layers has also been shown to correlate with the final visual outcome. These changes can be observed on SD-OCT and correlate with the visual acuity. Accurate evaluation of the second hyper reflective band in the macular region, the Ellipsoid Zone, on OCT is highly significant for providing valuable information regarding disease state and visual prognosis in diabetic retinopathy. We report on the most current and accepted information regarding this disorganization of inner retinal layers and outer retinal layers and its accurate clinical evaluation.*

Diabetic retinopathy is one of the leading causes of vision loss worldwide[1]. Optical coherence tomography (OCT) is a non-invasive tool which provides information about the in-vivo retinal histology. Diabetic retinopathy (DR) results in structural changes within the inner and outer retinal layers that can be observed by OCT imaging. These structural changes correlate with the severity of vision loss[2]. On spectral domain optical coherence tomography (SD-OCT), a good correlation has been observed between macular thickness parameters and visual acuity (VA)[3,4].

A significant association between disorganization of microstructural architecture of retina and impairment of VA has been observed in Diabetic Macular Edema (DME). Disorganization of retinal inner layers (DRIL) and disruption of outer retinal layers (ORL) contribute to significant decrease in VA in DME (Table 1).

DISORGANIZATION OF RETINAL INNER LAYERS

DRIL has emerged as an important parameter on SD-OCT in evaluation of DME. It is defined as the horizontal extent for which any boundaries between the four inner retinal layers are not identified. These layers include: ganglion cell–inner plexiform layer complex (GCL+ IPL), inner nuclear layer (INL) and outer plexiform layer (OPL) (Figure 1). It has been established as a non-invasive predictor of VA in eyes with center-involving and center-sparing DME.

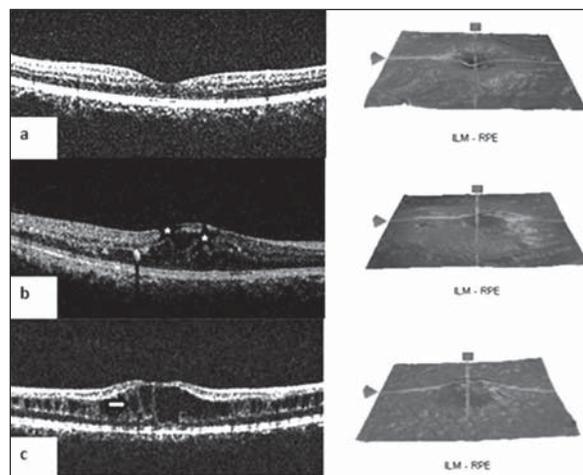| Table 1	
DRIL	**ORL**
Ganglion cell layer (GCL)	External limiting membrane (ELM)
Inner plexiform layer (IPL)	Ellipsoid zone (EZ)
Inner nuclear layer (INL)	Cone outer segment tips (COST)
Outer plexiform layer (OPL)	Retinal pigment epithelium (RPE)

Figure 1(a): *SD-OCT cross-sectional image shows normal OCT and ILM-RPE overlay map shows normal macular thickness.* **(b):** *SD-OCT cross-sectional image shows cystic spaces in inner retina (star) and ILM-RPE overlay map shows increased macular thickness.* **(c):** *SD-OCT cross-sectional image shows extensive cystic changes in inner retina (arrow) and ILM-RPE overlay map shows increased macular thickness.*

Various studies have been conducted in order to evaluate the association of changes in DRIL with VA. In one of the studies conducted by Sun et al, 1-mm-wide retinal area centered on the fovea was evaluated by masked graders for DRIL extent, cysts, hyper reflective foci, microaneurysms, cone outer segment tip visibility and external limiting membrane or photoreceptor disorganization and reflectivity. The study concluded that an early change in DRIL was associated with a substantial decrease in VA[5].

The correlation of DRIL with VA after the resolution of macular edema in diabetics was further established. Radwan et al conducted a study in which central 1500-μm macular region was analyzed for changes, including cysts, DRIL length and extent, and outer retinal layers disorganization.VA after DME resolution correlated with baseline VA. It was found that the patients whose DRIL resolved, both early and late, showed

improvement in their VA compared with non-resolvers, whose VA worsened. The study concluded that the presence of DRIL at baseline and its resolution pattern may be associated with subsequent VA improvement after resolution of center-involved DME[6].

The mean ganglion cell layer and mean retinal nerve fiber layer are also observed to be thinner in patients with DR as compared to patients with no DR. Thus, a significant thinning of different inner cell layers and the central retina is present in patients with early DR compared to normal eyes[7].

OUTER RETINAL LAYERS

Outer retina has four discrete bands. External limiting membrane (ELM) is the innermost layer present as a linear confluence of junctional complexes between Muller cells and photoreceptors. The ellipsoid zone (EZ) is the second hyper reflective layer. The third layer is the interdigitation zone (IZ) between cone outer segment tips and apical processes of Retinal Pigment Epithelium (RPE). The highly-reflective outermost band represents the RPE/ Bruch's complex[8,9].

In the year 2011, Spaide and Curcio highlighted that the second band initially described as inner segment-outer segment junction (IS-OS) of photoreceptor was principally the EZ of the photoreceptor[10]. With the cellular level resolution obtained by OCT, statistically significant relationship between VA and percentage disorganization of EZ has been observed. Furthermore, macular volume is also found to be significantly associated with visual acuity[11].

An association has been found between the status of the ELM prior to treatment and visual outcomes post treatment in pathologies like epiretinal membranes, age-related macular degeneration and DME[12-14]. ELM is considered as third blood barrier and is formed by zonula adherens and tight junctions. The shortening of the photo receptor inner segment might be a secondary consequence of the fragmented ELM, therefore disorganization of the ELM is noted earlier than EZ disorganization. Our earlier research has highlighted the importance of an intact ELM at the fovea for retinal photoreceptor microstructures integrity and visual acuity[15].

Our previous studies have also highlighted a positive correlation between the increase in serum vascular endothelial growth factor (VEGF) and
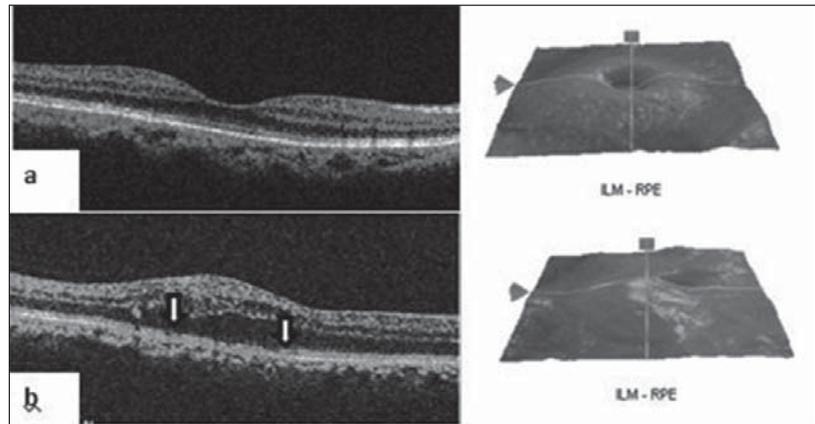

Figure 2(a): *SD-OCT cross-sectional image shows normal outer retinal layers in OCT and ILM-RPE overlay map shows normal macular thickness.* ***(b):*** *SD-OCT cross-sectional image shows disrupted ellipsoid zone in the outer retina (arrow) and ILM-RPE overlay map shows increased macular thickness.*

Intercellular adhesion molecule-1 (ICAM-1) levels with severity of diabetic retinopathy. A positive correlation was observed between the grades of ELM and EZ disruption and the levels of VEGF and ICAM-1. Also, the grades of ELM and EZ disruption significantly correlate with decrease in VA.

Recently, a significant correlation between the macular thickness parameters, disorganization of EZ and the increase in severity of diabetic retinopathy has also been documented (Figure 2). It was observed that severity of DR was associated with increase in disorganization of EZ. 'Global' disorganization of EZ was more prominent in proliferative diabetic retinopathy as compared to non-proliferative diabetic retinopathy. The decrease in VA was observed to be significantly associated with increased disorganization of EZ and severity of retinopathy[16].

FACTORS AFFECTING INTEGRITY OF ELLIPSOID ZONE

The advanced glycation end products lead to an increase in ICAM-1 and VEGF levels, which further cause an increase in the leucocyte adhesion to retinal endothelial cells and leads to breakdown of blood retinal barrier. Increase in the levels of ICAM-1 and VEGF results in EZ and ELM disorganization[17]. Increased oxidative stress (Plasma lipooxygenase, nitric oxide) is also associated with in-vivo structural changes in EZ[18].

RETINAL PIGMENT EPITHELIUM

Retinal pigmented epithelium (RPE) is a constituent of the outer blood retina barrier and is considered important for the maintenance of the integrity of retina.

An increase in the plasma levels of Lipid peroxide, Nitric oxide, EZ disorganization and RPE topographic alteration showed a significant association with increased severity of diabetic retinopathy. Single layer retinal pigment epithelial (SL-RPE) map has been used to evaluate the topographic alterations in RPE.

FACTORS AFFECTING TOPOGRAPHY OF RPE

RPE is responsible for transport of ions, retinal proteins, growth factors and metabolism of the photoreceptor layer. Increased nitric oxide levels are responsible for RPE damage and thus, cause breakdown of the Blood retinal barrier in diabetics. Neuronal retina and photoreceptors are the most affected intraocular tissues when RPE is damaged. Increased oxidative stress (nitric oxide and plasma lipooxygenase) was found to have a significant association with topographic alterations in RPE[19].

CLASSIFICATION SYSTEMS FOR OUTER RETINAL LAYER DISRUPTION

Various classification systems have been devised in DR to prognosticate visual acuity.

1. Classification system for ELM and EZ Disorganization was given by Jain et al
   i) Grade 0: no disorganization
   ii) Grade 1: ELM disrupted, EZ junction intact.
   iii) Grade 2: both ELM and EZ junction disrupted
2. In the study by Sharma et al, a simplified, comprehensive and physician-friendly approach of grading EZ disorganization was

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developed.

EZ is graded on horizontal and vertical scans as 'focal' and 'global'.

   I)   Grade 0: Intact EZ.

   II)   Grade 1: Focal disorganization (localized, subfoveal EZ disorganization)

   III) Grade 2: global disorganization (generalized EZ disorganization throughout the macular cube).

3. Study by Sharma et al proposed another classification system for RPE alterations in diabetic retinopathy.

   i)   Grade 0: No RPE alterations

   ii)   Grade 1: RPE alterations in up to two quadrants

   iii) Grade 2: RPE alterations in more than two quadrants.

CONCLUSION

DRIL and ORL are important predictors of VA in patients with Diabetic retinopathy. The integrity of outer and inner layers on OCT correlates well with final visual acuity and response to treatment in DME.

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