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# Association of Ellipsoid Zone, External Limiting Membrane Disruption and Disorganization of Retinal Inner Layers as Retinal Imaging Biomarkers by Spectral Domain Optical Coherence Tomography (SD-OCT) in Diabetic Macular Edema

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**Abstract:** ***Context**: There are several spectral domain optical coherence tomography biomarkers in diabetic macular edema. These are disorganization of the retinal inner layers (DRIL), ellipsoid zone and external limiting membrane disruption. Further studies are required to evaluate these retinal imaging biomarkers. **Aims**: To determine the role and advantages of these biomarkers and their association with visual outcome after treatment or normal follow up in patients of diabetic macular edema. **Settings and Design**: Prospective and interventional study was conducted from January 2021 to December 2021. **Methods and Material**: Optical coherence tomography scan was carried out in 50 eyes of 50 patients of diabetic macular edema out of which disorganization of retinal inner layers were found in eyes 28 eyes (56%) and ellipsoid zone and external limiting membrane disruption was found in 35 eyes(70%) and they were given 0.05 ml of ranibizumab monthly injection for consecutive 3 months. **Statistical analysis**: IBM.SPSS statistics software 23/0 version was used. To find the significance in categorical data Chi-square test was used and in the multivariate analysis the one way ANOVA with Tukey's Post-Hoc test was used. **Results**: In our study we found that ellipsoid zone , external limiting membrane disruption and horizontal extent of disorganisation of retinal inner layers directly proportional to the decrease in visual acuity($p=0.001<0.05$) , ($p=0.012<0.05$), ($p=0.006<0.05$) respectively also the horizontal extent of disruption had decreased after injecting 3 monthly intravitreal ranibizumab . In statistics probability value 0.05 is considered as significant level. **Conclusion**: Final visual outcome depends on these imaging biomarkers.*

**Keywords:** Disorganisation of retinal inner layers , Spectral domain optical coherence tomography, Ellipsoid zone and external limiting membrane disruption.

## 1. Introduction

Diabetes mellitus is one of the main causes of blindness in industrialized countries and also of severe loss of vision in the working population. Diabetes causes chronic disabling complications that affect mainly the eyes, kidneys, peripheral nervous system and cardiovascular system. As an ophthalmologist we are concerned with ocular changes.

30-50% of the diabetic population has retinopathy and every year 1% is affected by severe forms of the disorder[1]. The main risk factors associated with earlier onset and more rapid progression of diabetic retinopathy are: duration of diabetes, raised blood sugar levels and arterial hypertension. Diabetic retinopathy is one of the micro-circulatory lesions. It affects causes lesions in vessel wall, changes in blood flow, changes in platlets.

One of the important cause of vision loss in diabetic retinopathy patients is the development of diabetic macular edema. The **Wisconsin Epidemiologic Study of Diabetic Retinopathy (WESDR)**[2] found that 20% of patients with type 1 diabetes and 25% patients with type 2 diabetes will develop DME after 10 years of follow-up. In retina outer blood retinal barrier is formed by tight junction or zonula occludentes between RPE. Inner blood retinal barrier is formed by tight junctions between retinal capillary endothelial cells.

**(Lopes de Faria et al.1999)**[3]**; (Fong et al.2003)**[4] Macular edema is formed in all the stages of retinopathy, whether non-proliferative, moderate or severe or even at the more advanced stages of the retinopathy but it appears to occur more frequently as the severity of diabetic retinopathy increases. It is formed at the level of the outer plexiform layer with regard to interstitial /extracellular/vasogenic edema but also there is intracellular/cytotoxic edema. The causes of edema are therefore: congestion, osmotic factors and ischaemia.

**Klaassen et al (2013)**[5]they stated that in diabetic retinopathy the endothelium loses its role in blood retinal barrier so the capillaries becomes hyperpermeable and lose their resistance and the basement membrane of vessels thickens and pericytes are altered. Due to hyperpermeability of capillaries fluids leak through the altered walls thus forming edema. Diabetic macular edema leads to distortion of visual images and may cause a significant decrease in visual acuity even in the absence of severe retinopathy.

Retina is a very fragile membrane that is sustained by a complex framework of fibres, capillaries and cells organized in vertical and horizontal structures which forms anatomical and functional barriers. These vertical and horizontal structure explains localization, dimensions and shape of exudates, haemorrhages, cystoid cavities that we can analyze with optical coherence tomography.

Extension of pathological processes is blocked vertically and horizontally by these barriers. In the extra macular area the

retinal framework is quite simple. Muller cells unite the inner limiting membrane to the outer limiting membrane crossing the two plexiform layers. Macula has a high degree of complexity and its structure is weaker than retinal extra macular framework. Anatomy of the macula explains why cystoid edema is flower or honeycomb shaped. Pathological processes extension in the macular area is blocked vertically and horizontally by these barriers.

Methods for diagnosis of diabetic macular edema were stereoscopic diagnosis by fundus colour images and slit lamp biomicroscopy by 78/90 D lens but by these measures we were not able to assess retina layer by layer. Spectral domain optical coherence tomography provides better resolution and reproducibility so pathologies can be identified layer by layer. **Pateep Phadikar, Sandeep Saxena et al(2017)[6]:** According to them the word "biomarker" means biological biomarker which indicates the state of health and well being of an individual. There can be anatomical, biochemical, molecular and imaging biomarkers. They are measured by physical examination , by laboratory technique or by imaging technique.

**Sandeep Saxena,Grewal and Jaffe et al(2017)[7]** have used disorganization of the retinal inner layers as an optical coherence tomography biomarker in various causes of macular edema. Several trials are going on for this type of optical coherence tomography based study, so this study aiims to better understand and establish the role of optical coherence tomography in diabetic macular edema which will definitely help our patients in early their diagnosis and to monitor the treatment response. The lacunae in the knowledge is that there are very few international prospective and longitudinal studies available to determine the role of Disorganization of the retinal inner layers(DRIL), ellipsoid zone and external limiting membrane disruption as Spectral domain optical coherence tomography biomarker in diabetic macular edema. Therefore aim of the study is to determine the role of these biomarker in diabetic macular edema.

## 2. Methods

This is a prospective and interventional study conducted over a span of one year from January 2021 to December 2021 after taking permission from ethical committee and written informed consent was obtained from all the patients . Inclusion and exclusion criteria were as follows:

**Inclusion criteria**
Patients > 25 years of age of both genders, patients who were diagnosed with macular edema on screening patients of diabetes and have not undergone any type of treatment earlier.

**Exclusion criteria**
History of ocular trauma and surgery, advanced media opacities (cataract > grade2 Lens opacification and classification system), poor image quality on optical coherence tomography with signal strength 5 or below on spectral domain optical coherence tomography, Corneal scarring, other Retinal abnormalities which could affect retinal appearance (retinal detachment, macular hole, retinal vein occlusion, age related macular degeneration, abnormality of vitreo-retinal interface, vitreous haemorrhage, Hypertensive retinopathy, central serous chorioretinopathy), any previous surgical or laser interventions, myopia > 6D, history of Glaucoma and uveitis, patients on anticoagulant, contraceptive pills, patients with systemic diseases like end stage renal disease, nervous system (Alzheimers disease, peripheral neuropathy), cardiovascular disease, tuberculosis, chronic liver disease, hypertension were also excluded.

50 eyes of 50 patients of diabetic macular edema were scanned out of which disorganization of the retinal inner layers(DRIL) was present in 28 eyes(56%) and ellipsoid zone and external limiting membrane disruptionwas found in 35 eyes(70%) and they were given 0.05 ml of ranibizumab monthly injection for a consecutive 3 months.

All the patients have undergone detailed examination.

Best corrected Visual Acuity (Snellen chart),Slit lamp examination (zeiss)

Intra-ocular pressure measurement using Goldmann Applanation Tonometry.

Fundus examination: After pupillary dilatation ,fundus was examined with 78/90D lens by slit lamp biomicroscopy following which detail fundus examination was done with indirect ophthalmoscope, status of retinopathy were recorded and cases were divided into 2 groups based on early treatment of diabetic retinopathy study (ETDRS) classification into non proliferative diabetic retinopathy with macular edema and proliferative retinopathy with macular edema.

**Optical Coherence Tomography Scanning Protocols**

It was done using spectral domain optical coherence tomography (SD-OCT) [Cirrus High Definition OCT {Carl Zeiss MeditecInc., CA, U.S.A}] Dublin C.A , model 500, software version 6.5.0.772 using macular cube 512x128 and using 5 line raster scan with spacing of 0.25 mm, length = 6mm and scan angle of zero deg.Pupils were dilated before doing the imaging, central subfield thickness was noted as the average thickness of the central macular area. Central subfield thickness (CST), Cube average thickness (CAT) both in micrometers and cube volume (mm3) were noted. For disorganization of the retinal inner layers grading is done as follows:
Mild: less than 200 µm
Moderate: between 200 – 800 µm
Severe: above 800 µm
Grading of ellipsoid zone and external limiting membrane disruption of was done according to the **Anjali S.Maheshwary** in which

Grade 1: is intact that is when there is no disruption
Grade 2: focal disruption of 200 µm or less
Grade 3: disruption of more than 200 µm.In this study we have used horizontal extent of disorganization of the retinal inner layers 1000 - 3000 µm of the foveal area.

## 3. Results

The collected data were analysed with IBM.SPSS statistics software 23.0 Version. To describe about the data descriptive statistics frequency analysis, percentage analysis were used for categorical variables and the mean and standard deviation were used for continuous variables. To find the significant difference in the multivariate analysis the one way ANOVA with Tukey's Post-Hoc test was used. To find the significance in categorical data Chi-Square test was used. In all the above statistical tools the probability value .05 is considered as significant level.

## 4. Discussion

In our study we found that if the mean value of central subfield thickness increases , horizontal extent of disorganization of retinal inner layers also increases and extent of disorganization of retinal inner layers is more in the proliferative stage of diabetic retinopathy when compared with the non proliferative stage. Mean value of central subfield thickness in our study was 383.1 µm when the disorganization of retinal inner layers was absent and when the disorganization of retinal inner layers was present mean value of central subfield thickness was 451.3 µm. This is in accordance with study done by **Sandeep Saxena et al**[8] so our findings is consistent with those from the previous studies. However in our study we found that mean value of central subfield thickness was slightly higher in mild disorganization of retinal inner layers as compared with that of moderate and severe disorganization of retinal inner layers. The reason for this could be discrepancy in the sample size that is in the severe disorganization of retinal inner layers there were 4 patients, in mild 8 patients and in moderate 16 patients were there.

In this study after giving intravitreal injection in about 48% of the eyes disorganization of retinal inner layers had resolved completely whereas in 36% of patients it had become less than 200µm and in about 16% of patients it had become in the range of moderate disorganization of retinal inner layers. We found that in eyes which had little change in disorganization of retinal inner layers extent had also little change in visual acuity despite resolution of macular edema and reduction in central subfield thickness . This is in according to the study done by **Radwan H.Salma et al**[9]in which they concluded that eyes in which disorganization of retinal inner layers was resolved showed improvement in their visual acuity at 8 months of follow up as compared with non-resolvers in whom visual acuity had worsened. The probable reason for this worsening of vision could be that there is a particular value above which if the edema will occur there will be no improvement in vision because if the edema will exceed the particular value then there will be disarrangement in the inner retinal layers which leads to breakdown of the neural synaptic connection between the amacrine cells, horizontal cells and bipolar cells, so this process is responsible for irreversible loss of visual acuity.

Also in our study we found incidentally in 2 eyes that if no intervention was done (patient refused for intervention) then there was increase in the disorganization of retinal inner layers extent over a period of month which means that chronicity plays an important role which is in accordance with the study done by **Shah et al**[10] in which they stated that " bipolar cells may get stretched beyond their limits which prevents transmission of signals from photoreceptors to ganglion cells and this is responsible for poor visual acuity."

In our study we found that if the external limiting membrane, ellipsoid zone disruption is in grade 3 then mean central subfield thickness is around 462.9 µm and 458.9 µm respectively and visual acuity is less than 6/60i,e. more the impairment of external limiting membrane on both horizontal and vertical scan, higher will be the central subfield thickness value and lesser will be the visual acuity and vice-versa. So the value of central subfield thickness is directly proportional to the external limiting membrane and ellipsoid zone disruption length i.e higher the value of central subfield thickness, higher is the grade of external limiting membrane and ellipsoid zone . This is very similar to the prospective study done by **Saxena S,K.Srivastav et al**[11].

This finding is also consistent with the retrospective study done by **Shin-ichiro et al**[12].In the same way retrospective study done by **Anjali S et al**[13] have found that more the impairment of ellipsoid zone in both horizontal and vertical scan, lesser will be the visual acuity and vice-versa.

In this study we also found that higher the stage of diabetic retinopathy (ETDRS classification), larger will be the impairment of external limiting membrane, ellipsoid zone in both horizontal and vertical scans. During analysis we found that about 50% of proliferative diabetic retinopathy (PDR) eyes had grade 3 disruption of external limiting membrane. This is in accordance with the prospective study done by **Saxena S, Sharma SR et al**[14]in which they found that with increase in severity of diabetic retinopathy disruption of ellipsoid zone also increased ($r = 0.49$, $p < 0.001$). Also 'Global' disruption of ellipsoid zone in proliferative diabetic retinopathy (PDR) group was significantly higher as compared with non proliferative diabetic retinopathy (NPDR). Decrease in visual acuity was also found to be significantly greater with increased disruption of ellipsoid zone and severity of retinopathy. This is similar to the cross-sectional study performed by **SukritiAhuja,SaxenaS et al**[15]in which they concluded that with increase in severity of diabetic retinopathy ellipsoid zone disruption also increases.

After giving intravitreal injection we found that the integrity of ellipsoid zone and external limiting membrane had improved and also there was an improvement in visual acuity. This is in according to **VISTA study** done by **Ehlers JP et al**[16] in which after giving 5 monthly injection of anti-VEGF there was an improvement in ellipsoid zone integrity.

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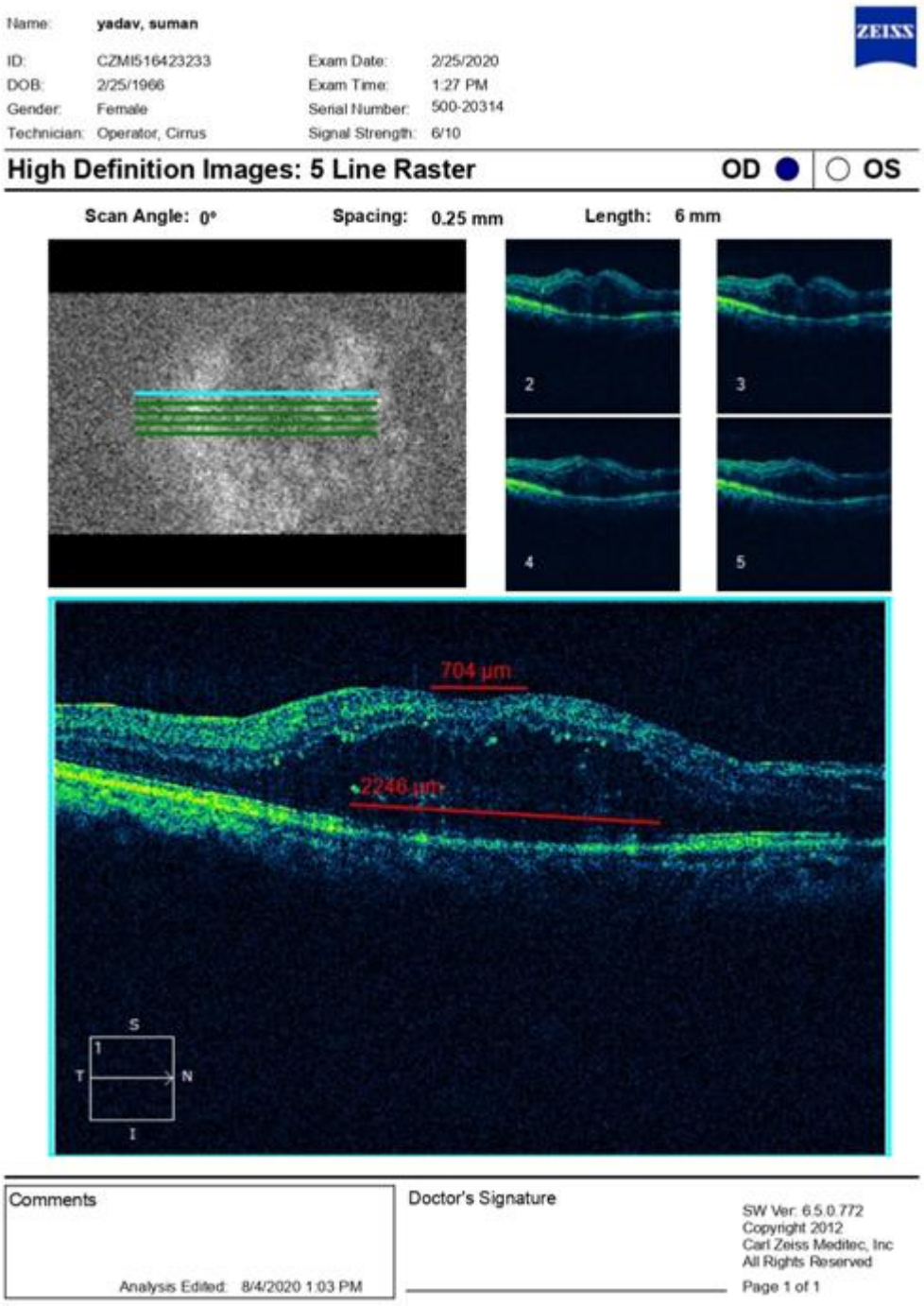


**Figure 1:** DRIL extent= 704 microns
ELM and EZ disruption length= 2246 microns

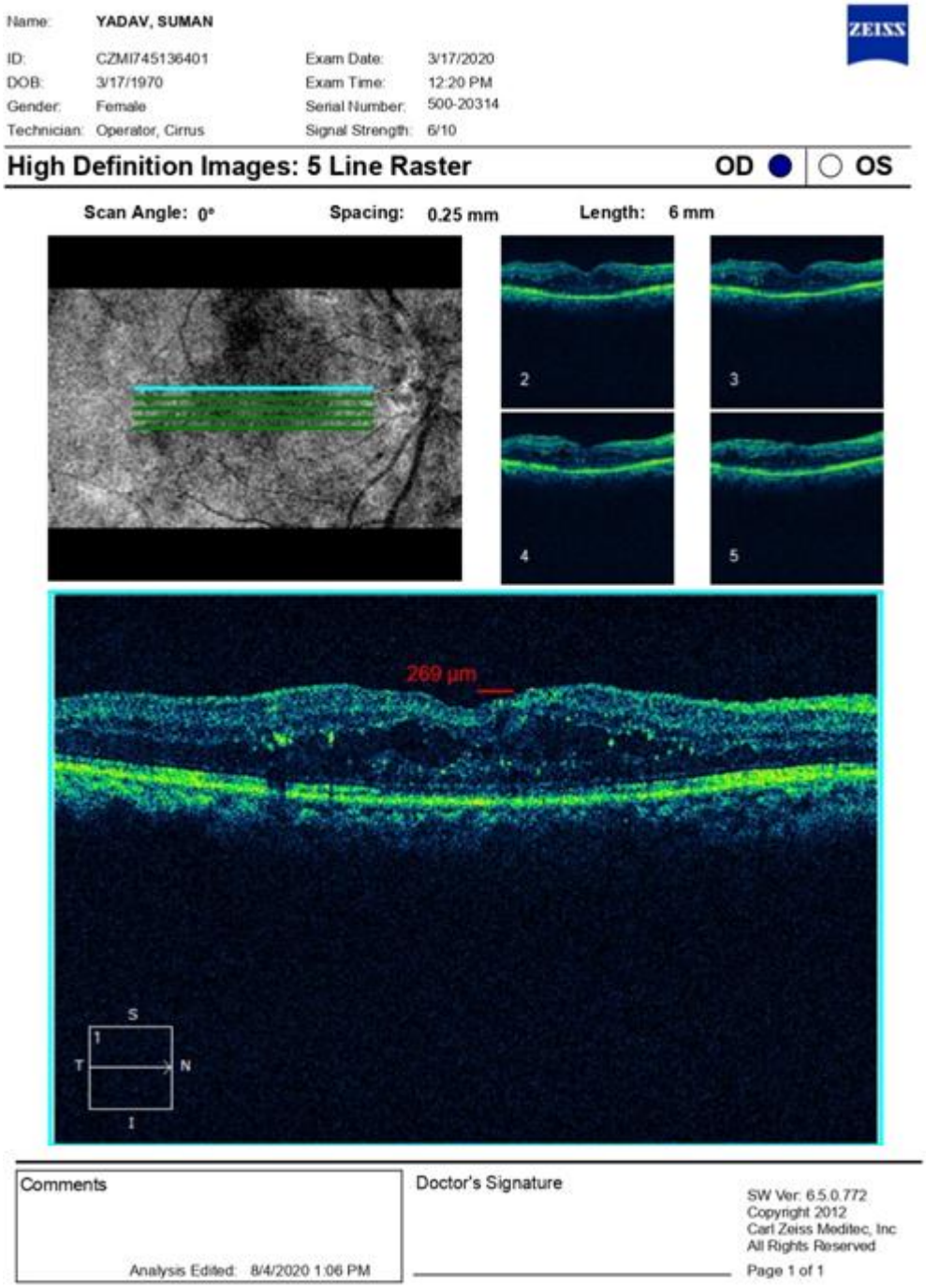


**Figure 2:** Same patient as above after intravitreal anti – VEGF DRIL extent reduces to 269 microns with resolution of ELM and EZ disruption.