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CASE REPORT

Pathogenic Mechanisms for Outer Retinal Layer Changes in Diabetic Retinopathy

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ABSTRACT

Diabetic Retinopathy, primarily a disease of the retinal vasculature, is a major public health problem, being responsible for more irreversible blindness than any other pathology in middle and older age people. The underlying pathogenic mechanisms are not clearly understood.

In this study we aim at studying the role of Intercellular Adhesion Molecule (ICAM-1) and its interaction with Vascular Endothelial Growth Factor (VEGF), Advanced Glycation End products (AGEs) and oxidative stress in the pathogenesis of diabetic retinopathy and use of Spectral Domain Optical Coherence Tomography (SD OCT) as a tool in diagnosing, evaluating and monitoring progression of DR on the basis of pathological changes in outer retinal layers and its association with visual outcome .The study will additionally describe novel biochemical and imaging biomarkers and there correlation with progression of diabetic retinopathy .

INTRODUCTION

Diabetes Mellitus is one of the most common metabolic disorders and one of the leading causes of morbidity worldwide. According to International Diabetic Federation (IDF) 2014 report, 387 million people were afflicted with diabetes in 2014 across the globe, an alarming number that is set to cross 592 million by 2034. A further 316 million with impaired glucose tolerance are at high risk from the disease, with projections indicating that over 1 billion people will be living with or at high risk of diabetes in 2035 [1].

Primarily a disease of the retinal vasculature, diabetic retinopathy reflects a dysfunction of the metabolic, endocrine and hematological systems. It is an important cause of blindness. In 2010, it was estimated that 285 million people worldwide had diabetes. Among them, over one-third have signs of DR, and a third of these are having Vision-Threatening Diabetic Retinopathy (VTDR), defined as severe non-proliferative DR or proliferative DR (PDR) or the presence of Diabetic Macular Edema (DME) [2].

Although pathogenesis of diabetic retinopathy is not very well understood, a number of risk factors have been implicated that includes poor glycemic control, duration of diabetes mellitus, increasing age, hypertension, dyslipidemia, serum urea and serum creatinine [3-5].
Diabetic Retinopathy is classified into two types depending on the severity and clinical appearance:

1. Non Proliferative Diabetic Retinopathy (NPDR)

It is the early stage of the disease. Symptoms will be mild or non-existent. NPDR is characterized by microaneurysms, intraretinal hemorrhages, cotton wool spots, hard exudates, venous beading and intraretinal microvascular abnormalities. In NPDR, retinal blood vessels become weak causing micro aneurysms. The micro aneurysms may leak into the retina, which may lead to swelling of the macula.

2. Proliferative Diabetic Retinopathy (PDR)

This is the more advanced form of disease. It is characterized by neovascularization of retina and disc vessels and their sequel. At this stage, circulation problems cause the retina to become deprived of oxygen leading to growth of new blood vessels in the retina and into the vitreous, which being fragile may leak blood into the vitreous or retina. Other complications of PDR include retinal detachment due to scar and development of glaucoma. Proliferative diabetic retinopathy can cause severe vision loss and even blindness, if left untreated.

SPECTRAL DOMAIN OPTICAL COHERENCE TOMOGRAPHY IN DIABETIC RETINOPATHY

Spectral Domain (SD-OCT) is a widely accepted imaging modality now days. It is a non contact non invasive technique which captures the real time in vivo images of retinal microstructures using

the principle of low coherence interferometry. It has made a significant impact in the diagnostic evaluation of patients with DR [6]. It provides cross sectional and three dimensional (3D) views of retina. Its role in evaluating the status of macula with progression of diabetic retinopathy is well established.

CURRENT CONCEPTS IN PATHOGENESIS OF DIABETIC RETINOPATHY

Intercellular Adhesion Molecule (ICAM-1) that is necessary for the adhesion of leucocytes to the capillary endothelium is a member of the immunoglobulin super family [7]. A prominent feature of diabetic retinopathy is development of leukostasis, which is mediated by ICAM-1 [8]. Leukocytes adhere to the retinal vascular endothelium before any clinical pathology is apparent [9]. In diabetes their occurs increased expression of ICAM-1, and its specific inhibition prevents diabetic retinal leukocyte adhesion and breakdown of blood-retinal barrier [10]. ICAM-1 is shed by the cell and detected in plasma as sICAM-1. ICAM-1 is the key moderator of the effect of Vascular Endothelial Growth Factor (VEGFs) on retinal leukostasis [11].

VEGFs are key regulators of vascular development during vasculogenesis and angiogenesis [12]. Hypoxia leads to VEGF-induced ocular neovascularization [13]. The imbalance between VEGF and angiogenic inhibitors leads to ocular neovascularisation in diabetic retinopathy [14]. VEGF is involved in the commencement of retinal vascular leakage and non-perfusion in diabetes.

Advanced Glycation End products (AGEs) are non enzymatically glycated and oxidized proteins or lipids. They accumulate in the vessel wall and lead to disturbance in vascular endothelial and pericyte cell structure and function [15-18]. Formation of AGEs leads to accumulation of lipofuscin and reduction of lysosomal degradative capacity in RPE cells that in turn leads to impaired degradation of engulfed photoreceptor remnants [19]. Hyperglycemia results in various retinal biochemical alterations that is activation of polyol pathway, advanced glycation end products accumulation and diacyl glycerol/protein kinase C activation leading to reactive oxygen species formation which in turn leads to increased expression of various growth factors and cytokines like TNF-α and nuclear factor KB (NF-kB) [20,21]. Increased activation of transcription factor NF-kB leads to increased expression of pro-inflammatory mediators inciting a vicious cycle of inflammation [22-26]. In addition to being an integral component of the innate immune response, MPO-derived oxidants contribute to tissue damage during inflammation [27,28].

Retina has 10 layers. Outer retina has four discrete bands, a linear confluence of junctional complexes between Muller cells and photoreceptors [29] labelled as External Limiting Membrane (ELM), is the inner most layer. The second hyperreflective layer correlates to the Ellipsoid Zone (EZ) [30,31]. The third layer correlates to the interdigitation zone between cone outer segment tips and apical processes of Retinal Pigment Epithelium (RPE). The outermost highly-reflective zone represents the RPE/Bruch's complex [32]. Clinical relevance to the subject can be attached with ELM, EZ and RPE.

1. External limiting membrane (ELM)

A association between the status of the ELM prior to treatment and visual outcomes post treatment has been described for epiretinal membranes [33-35] age-related macular degeneration [36] and diabetic macular edema [37]. Although the presence of undisturbed ELM was thought to be having a positive correlation with visual outcome in these diseases [37-39], there was only a weak correlation between the length of ELM at the fovea prior to treatment and visual outcomes post-treatment. In AMD patients, shorter pre-treatment ELM length was associated with a lesser degree of change in VA post treatment, while longer ELM length did not did not have any significant association with visual improvement [38]. In ERM eyes, no statistically significant association between the preoperative length of ELM and postoperative VA was found [35,36].

Our earlier studies discovered and established ELM as part of retinal barrier. It was highlighted that ELM disrupts first leading to EZ disruption and loss of photoreceptor integrity. Retinal photoreceptor disruption was defined as a predictor of visual acuity and progression of diabetic retinopathy. Our novel research suggests that an intact ELM at the fovea is necessary for retinal photoreceptor microstructures integrity and visual acuity.

An increase in serum VEGF and ICAM-1 levels were found to have a positive correlation with severity of diabetic retinopathy and the grade of ELM and IS-OS junction disruption [38].

2. Ellipsoid Zone

Fernandez et al divided the Inner-segment of photoreceptors into ellipsoid and myoid segments, using ultra high resolution SD-OCT images of human foveal cone photoreceptors. Spaide and Curcio established that the second band earlier reported as Inner Segment -Outer Segment junction (IS-OS) of photoreceptor was basically the EZ of the photoreceptor.

According to Multivariate analysis ,there is a statistically significant correlation between the visual acuity and degree of EZ disruption based on cellular level resolution obtained by SD OCT. Lately, significant correlation between macular thickness parameter and disruption of EZ with increasing severity of diabetic retinopathy has also been documented [39].

CLASSIFICATION SYSTEMS FOR ELLIPSOID ZONE AND EXTERNAL LIMITING MEMBRANE DISRUPTION

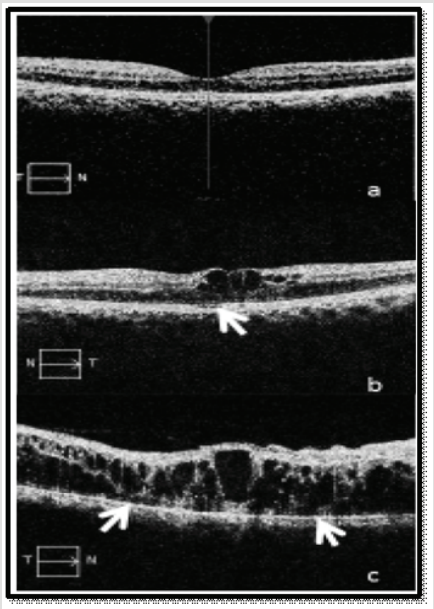

Figure 1: SD OCT macular cube showing EZ.
a: Grade 0: intact EZ
b: Grade 1: focal disruption (localized, subfoveal EZ disruption)
c: Grade 2: global disruption (generalized EZ disruption throughout the macular cube).

A. Sharma et al, put forward a simplified, comprehensive and physician-friendly approach to grading EZ disruption based on OCT of macula:

- Grade 0: intact EZ
- Grade 1: focal disruption (localized, subfoveal EZ disruption)
- Grade 2: global disruption (generalized EZ disruption throughout the macular cube)

The grade of subfoveal ELM and EZ disruption was with increase in severity of diabetic retinopathy and decrease in visual acuity. 'Global' EZ disruption was associated with marked decrease in visual acuity as compared to 'focal' disruption [40] (Figure1).

B. Jain et al gave the grading system of disruption of ELM and EZ in patients with diabetic retinopathy

EZ and ELM band disruption were graded as follows [40] (Figure 3):

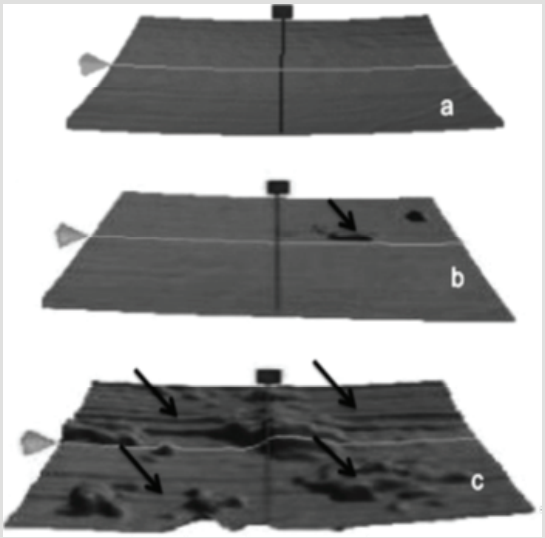

Figure 3: SD OCT macular cube showing RPE.
a: Grade 0: no RPE alteration
b: Grade 1: focal (alteration predominantly in up to two quadrants of the map)
c: Grade 2: global (alteration in more than two quadrants of the map)

- Grade 0: No disruption of ELM and EZ
- Grade 1: ELM disruption, intact EZ
- Grade 2: Both ELM and EZ disrupted

Grade of ELM and EZ disruption was related with decrease in VA and progression of diabetic retinopathy (Figure 2).

C. Retinal pigment epithelium

Interaction between photo-sensitive cells and the RPE is responsible for maintenance of healthy retina. The RPE is a constituent of the outer Blood–Retinal Barrier (BRB). VEGF that is an angiogenic factor and PEDF (pigment epithelium derived factor) that is an antiangiogenic factor is secreted by RPE. However, in diabetes mellitus, increased advanced glycation end products (AGEs), acts on RPE and endothelial pericytes, thus stimulate VEGF expression [41,42]. This increased expression of VEGF

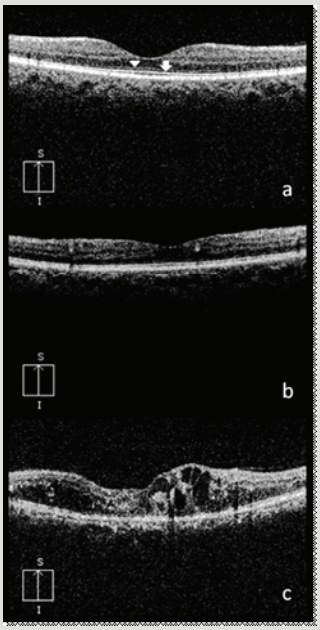

Figure 2: SD OCT macular cube showing ELM (arrow head) and EZ (arrow).
a: Grade 0: No disruption of ELM and EZ
b: Grade 1: ELM disruption, intact EZ
c: Grade 2: Both ELM and EZ disrupted

leads to breakdown of outer blood retinal barrier [43]. In conjuction, there is decreased expression of PEDF due to elevated glucose concentration. Thus, balance between VEGF and PEDF expression by RPE is crucial for progression of disease and the VEGF secreted by RPE is important to maintain the structural integrity of the outer retina and choriocapillaris [44,45].

Increase in plasma levels of Lipid peroxide, NO and decrease in plasma levels of GSH, ISel disruption and RPE topographic alteration was significantly associated with increased severity of diabetic retinopathy [46].

Single layer retinal pigment epithelial (SL-RPE) map can be used to evaluate the topographic alterations in RPE. Alteration of this layer have been graded into three groups [47].

Grade 0: no RPE alteration
Grade 1: focal (alteration predominantly in up to two quadrants of the map)
Grade 2: global (alteration in more than two quadrants of the map)
(Figure 3 and 4)

ROLE OF BIOMOLECULAR BIOMARKERS

Biomarkers: WHO defines 'Biomarkers' as objective, quantifiable characteristics of a biological process, pathogenic process, or a pharmacologic response to a therapeutic intervention.

Several biochemical and imaging biomarkers have been identified that have effect on progression of diabetic retinopathy. These are as follows:

1. Intercellular adhesion molecule and vascular endothelial growth factor

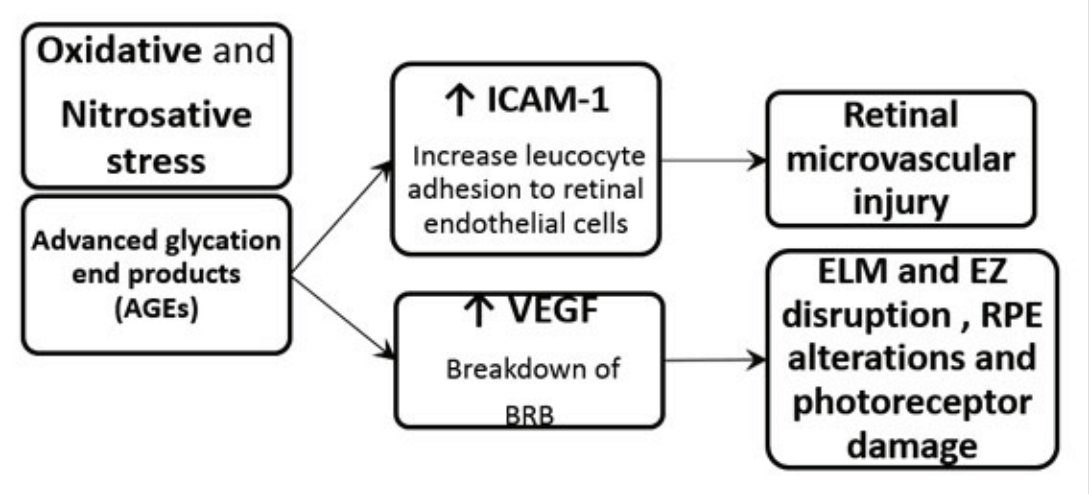

Figure 4: Schematic representation of pathogenesis of diabetic retinopathy.

Jain et al [48] demonstrated that serum levels of VEGF and ICAM-1 increased significantly with the severity of diabetic retinopathy. Increased levels of ICAM-1 leads to leukostasis which leads to vascular endothelial damage with formation of acellular capillaries. This results in retinal ischemia and up regulation of VEGF. Increase in VEGF in turn causes retinal neovascularization. The amount and duration of VEGF exposure required for blood-retina barrier breakdown is less than that required for neovascularization .Thus, elevated levels of ICAM-1 and VEGF come into play even before the signs of PDR have set in. The damage caused by them increases as the duration of the disease increases.

2. Anti- Myeloperoxidase Antibody

It has been described that diabetes is a state of chronic subclinical inflammation that is characterized by neutrophil activation. There is increasing evidence that suggests that chronic, subclinical inflammation plays an important role in the pathogenesis of DR.

Study conducted by Sinha et al [49] demonstrated that serum anti-MPO antibody increases as diabetic retinopathy progresses from non-proliferative to proliferative stage.

Myeloperoxidase (MPO), a pro-oxidant enzyme is stored in azurophilic granules of polymorph nuclear neutrophils and macrophages and in inflammatory conditions is released into extracellular fluid [50,51]. MPO by generating antimicrobial oxidants, free radicals and other reactive oxidant species protects against infections, this activity can also lead to oxidative damage of endothelium and vessel wall [52,53]. MPO leads to diminished nitric oxide bioavailability by directly scavenging nitric oxide which results in endothelial dysfunction [54-56]. Activated neutrophils releases factors that activate the alternative complement pathway, which further amplifies the inflammatory events through neutrophil recruitment and activation resulting in a vicious cycle [57].

3. N carboxy methyl lysine (an advanced glycation end product)

Nε-CML, the most prevalent AGE, is a biomarker of DR [58]. Interaction between photoreceptors and the RPE is required for maintenance of a healthy retina. Major contributor to retinal microvascular complication in type 2 DM is process of formation and accumulation of AGEs [59].

Study conducted by Mishra et al demonstrated, that as the levels of Nε-CML increases grades of RPE alterations in diabetic retinopathy also increases.

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