Retinal Pigment Epithelium Alterations in Diabetic Macular Edema

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Introduction

Diabetes mellitus will be the seventh leading cause of death in 2030 as projected by WHO.[1] The prevalence of Diabetic Retinopathy (DR) is intimately linked to the upsurge in prevalence of diabetes.[2] DR is the leading cause of vision loss in adults aged 20–74 years.[3] Proliferative diabetic retinopathy (PDR) is the most common vision-threatening lesion particularly among patients with type 1 diabetes mellitus. However, diabetic macular edema (DME) is responsible for most of the visual loss experienced by patients with diabetes as it remains the major cause of vision loss in the highly prevalent type 2 diabetes.[4,5]

RETINAL PIGMENT EPITHELIUM

ROLE OF RPE IN DEVELOPMENT OF RETINA

Healthy RPE has functional intact tight-junctions which are required for the effective and controlled removal of fluid from the subretinal space.[6] A variety of growth factors secreted by RPE maintain the structural integrity of choriocapillaris endothelium and photoreceptors. These growth factors are essential for both endothelial cell differentiation and photoreceptor differentiation.[7-11] RPE is essential for the maintenance of photoreceptor excitability. This is achieved by various mechanisms: stabilizing ion composition in the subretinal space,[12-14] phagocytosis of shed photoreceptor outer segments [15-18] and rebuilding light-sensitive outer segments from the base of the photoreceptors. Hence, RPE is essential for both development of retinal structures and visual function.[19]

PATHOPHYSIOLOGICAL CHANGES TAKING PLACE IN RETINAL PIGMENT EPITHELIUM IN DME

EFFECT OF VEGF ON RPE AND ITS RELATION WITH SEVERITY OF DR

Vascular endothelial growth factor (VEGF) is part of a subfamily of growth factors involved in angiogenesis. VEGF is secreted from retinal pigment epithelium (RPE) cells, pericytes, astrocytes, muller cells, glial cells and endothelial cells .[20] VEGF causes retinal capillary endothelium damage due to basement membrane thickening, pericyte loss and decreased capillary perfusion. This leads to fluid leakage out of the capillaries resulting into DME.

Serum VEGF levels serve as a simple, reliable, physician-friendly, and easy to comprehend biomolecular bio-marker for severity of DR. Significantly elevated levels of VEGF come into play even before the evidence of DR. Estimation of serum VEGF is a useful laboratory test for predicting the onset of DR.

VEGF has been shown to induce functional changes in the RPE.[21,22] Damage to RPE leads to degeneration of the retinal layers and impaired visual function.

In our earlier study we discovered that severity of DR was associated with increase in topographic RPE alterations (RPE-A) on SD-OCT .[23]

Clinical and OCT parameters and RPE grade showed significant increase in severity of diabetic retinopathy with increase in levels of Serum VEGF

EFFECT OF OXIDATIVE AND NITROSATIVE STRESS ON RPE

Increased NO-mediated damage was demonstrated and proposed to be responsible for the RPE damage and thus breakdown of the BRB in diabetic animals.[24] NO has been shown to decrease rod outer segment phagocytosis by RPE cells.[25] Exogenous NO has also been shown to inhibit human RPE cell proliferation.[26] RPE has been found to be responsible for transport of ions, retinal proteins, growth factors and metabolism of the photo receptor layer.[27,28] In case of damage of the RPE, neuronal retina and photo receptors are the most affected tissues in theeye.[29]

In our previous study we found that, with increase dseverity of diabetic retinopathy, increased levels of plasma NO and LPO were found to be significantly

related to decrease in visual acuity, disruption of the photo receptor ellipsoid zone and topographic alterations in RPE. Increased plasma NO levels were associated with RPE alterations.

ASSOCIATION OF RPE ALTERATIONS WITH DAMAGE TO BLOOD VESSELS AND CHANGES IN RESISTIVE INDEX

Blood Supply of RPE: Choriocapillaries which in turn are supplied by Ophthalmic artery through short posterior ciliary arteries

The integrity of the choroidal capillaries is regulated by RPE. Increased severity of DR, an increase in RI of OA results in decrease in blood flow to RPE. This leads to RPE topographic alterations and resultant decrease in VA.

Retinal capillary endothelium damage in diabetes occurs

due to basement membrane thickening, pericyte loss, increased expression of intercellular adhesion molecule-1 (ICAM-1),[30] advanced glycation end products (AGEs),[31] oxidative and nitro sative stress[32]and decreased capillary perfusion. This in turn leads to fluid leakage out of the capillaries resulting into DME, capillary closure and decreased capillary blood flow. In addition, positive correlation of AGEs with grades of RPE alterations has been observed in diabetic retinopathy.[33] Blood supply to retina is decreased due to biochemical and biomolecular changes with resultant retinal ischemia and increased vascular endothelial growth factor (VEGF) release .[34-36]

Blood flow to adjacent retinal capillaries is increased due to retinal ischemia, resulting in increase in vessel wall shear stress.[37] Capillary closure and alterations in rheological properties of blood also results in increased shear stress. Locking of the vessel occurs due to increased glycation and thickening of the basement membrane.[38] Also in the presence of dilated vasculature the systemic blood pressure is more easily transmitted to the micro circulation resulting in increased capillary pressure. As a result shear stress in vessel wall increases as the vessel diameter is unable to change, leading to mechanical injury to the vascular endothelium. This circumferential stress resulting into mechanical damage to the endothelium is directly proportional to the perfusion pressure and radius and inversely proportional to the thickness of the vessel wall.[39] Hence, circumferential stress damage occurs more on vessel with larger diameter resulting into further dilatation of vessel. The tension resisting circumferential stress in the vessel wall has an inverse relationship with the radius of the vessel, as a result tension to counteract circumferential stress is not attained in a dilated vessel, and therefore there is a tendency towards dilatation with consequent hyperperfusion.

Additionally, several other factors resulting in hyperperfusion are abnormal autoregulation of the retinal circulation,[40] increased conductance as an autoregulatory response to retinal ischemia,[41] endothelin-1 resistance, inhibition of calcium influx channel in smooth muscle cells and increased activity of nitric oxide synthase. As these changes occur in retinal vasculature the resistive index increases. In the present study, we found an increase in RI of OA with severity of DR, grades of RPE alterations and EZ disruption.

CORRELATION OF VITAMIN D WITH RPE ALTERATIONS

Normal serum Vitamin D levels range from 25 to 50 ng/ml. In our earlier study we found that the mean Vitamin D levels decrease with severity of DR. Low Vitamin D levels have also been found to correlate with increase in RPE-A.[42]

RPE TOPOGRAPHY BY SD OCT

Retinal Pigment Epithelium (RPE), the outermost hyperreflective band on SD-OCT is located between light-sensitive outer segments of the photoreceptors and choriocapillary vessels. RPE- Alterations (RPE-A) was evaluated by single layer retinal pigment epithelial (SL-RPE) map. RPE-A was graded as, Grade 0: No alterations, Grade 1: Alteration in two quadrants, Grade 2: Alteration in more than two quadrants12.

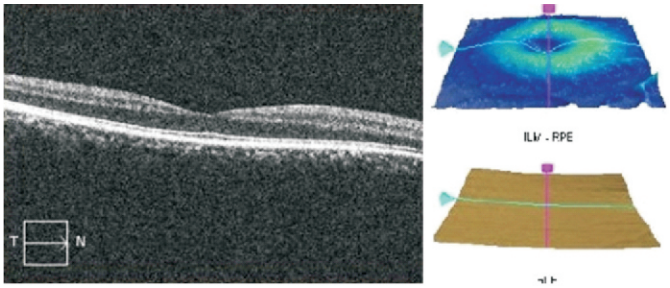

Figure 1a : SD-OCT showing normal RPE.

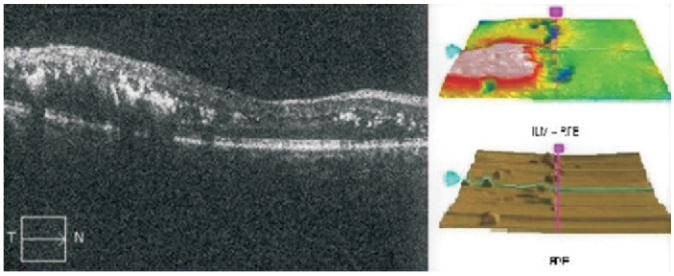

Figure 1b : SD-OCT showing DME on cross-sectional image, increased macular thickness on colour coded map and grade 2 RPE-A on RPE map.

TAKE HOME MESSAGE

1. **SD-OCT changes correlate significantly with severity of diabetic retinopathy and RPE-A. Hence must be performed at appropriate intervals for monitoring of the disease**
2. **There is positive correlation between VEGF levels and RPE-A.**
3. **Serum levels of Vitamin D correlate with increased severity of Diabetic retinopathy and RPE-A. Patients should be screened for Vitamin D deficiency.**

References

1. Mathers CD, Loncar D. Projections of global mortality and burden of disease from 2002 to 2030.. PLoS Med, 2006, 3: 442.
2. Yang W, Lu J, Weng J, Jia W, Ji L, Xiao J, et al. Prevalence of diabetes among men and women in China. N Engl J Med. 2010; 362:1090–101.
3. Cheung N, Mitchell P, Wong TY. Diabetic retinopathy. Lancet. 2010; 376(9735):124–36.
4. Lightman S, Towler HM. Diabetic retinopathy. Clin Cornerstone. 2003; 5:12–21.
5. Tong L, Vernon SA, Kiel W, Sung V, Orr GM. Association of macular involvement with proliferative retinopathy in type 2

diabetes. Diabet Med. 2001; 18: 388–94.
6. Marmor MF. Mechanisms of fluid accumulation in retinal edema. Doc Ophthalmol.1999; 97(34): 239–249.
7. Adamis AP, Shima DT, Yeo KT, et al. Synthesis and secretion of vascular permeability factor/vascular endothelial growth factor by human retinal pigment epithelial cells. BiochemBiophys Res Commun. 1993; 193: 631–8.
8. Behling KC, Surace EM, Bennett J. Pigment epithelium-derived factor expression in the developing mouse eye. Mol Vis. 2002; 8: 449–54.
9. Burns MS, Hartz MJ. The retinal pigment epithelium induces fenestration of endothelial cells in vivo. Curr Eye Res. 1992; 11: 863–73.
10. Ablonski MM, Tombran-Tink J, Mrazek DA, et al. Pigment epithelium-derived factor supports normal development of photoreceptor neurons and opsin expression after retinal pigment epithelium removal. J Neurosci. 2000; 20: 7149–57.
11. King GL, Suzuma K. Pigment-epithelium-derived factor: a key coordinator of retinal neuronal and vascular functions. N Engl J Med. 2000; 342: 349–51.
12. Dornonville DLCM. Ion transport in the retinal pigment epithelium: a study with double barrelled ion-selective microelectrodes. Acta Ophthalmol Suppl 1993; 209:1–32.
13. Steinberg RH. Interactions between the retinal pigment epithelium and the neural retina. Doc Ophthalmol. 1985; 60: 327–46.
14. Steinberg RH, Linsenmeier RA, Griff ER. Three light-evoked responses of the retinal pigment epithelium. Vis Res.1983; 23: 1315–23.
15. Bok D. The retinal pigment epithelium: a versatile partner in vision. J Cell Sci Suppl 1993; 17: 189–95.
16. Finnemann SC. Focal adhesion kinase signalling promotes phagocytosis of integrin-bound photoreceptors. EMBO J. 2003; 22: 4143–54.
17. Gal A, Li Y, Thompson DA, et al. Mutations in MERTK, the human orthologue of the RCS rat retinal dystrophy gene, cause retinitis pigmentosa. Nat Genet. 2000; 26: 270–1.
18. Strauss O, Stumpff F, Mergler S, et al. The Royal College of Surgeons rat: an animal model for inherited retinal degeneration with a still unknown genetic defect. Acta Anat. 1998; 162: 101–11.
19. Khatri, M., Saxena, S., Kaur, A. et al. Resistive index of ophthalmic artery correlates with retinal pigment epithelial alterations on spectral domain optical coherence tomography in diabetic retinopathy. Int J Retin Vitr 2018; 4, 12.
20. Gupta N, Mansoor S, Sharma A, et al. Diabetic retinopathy and VEGF. Open Ophthalmol J. 2013; 7: 4–10.
21. Hartnett ME, Lappas A, Darland D, et al. Retinal pigment epithelium and endothelial cell interaction causes retinal pigment epithelial barrier dysfunction via a soluble VEGF-dependent mechanism. Exp Eye Res.2003; 77(5): 593–599.
22. Zech JC, Pouvreau I, Cotinet A, et al. Effect of cytokines and nitric oxide on tight junctions in cultured rat retinal pigment epithelium. Invest Ophthalmol Vis Sci.1998; 39(9): 1600–1608.
23. Mishra N, Saxena S, Ruia S, et al. Increased levels of N(ε)-Carboxy methyl lysine (N(ε)-CML) are associated with topographic alterations in retinal pigment epithelium: A preliminary study. J Diabetes Complications. 2016; 30(5): 868-72.
24. Pacher P, Obrosova I, Mabley J, Szab ó C. Role ofnitrosative stress and peroxynitrite in the pathogenesisof diabetic complications, emerging new therapeuticalstrategies. CurrMed Chem 2005; 12: 267 – 75.
25. Becquet F, Courtois Y, Goureau O. Nitric oxidedecreases in vitro phagocystosis of photoreceptor outersegments by bovine retinal pigmented epithelial cells.J Cell Physiol 1994; 159: 256 – 62.
26. Yilmaz G, Esser P, Kociek N, Heimann K. Effect ofnitric oxide on proliferation of human retina pigmentepithelial cells. Eye 2000; 14: 899 – 902.
27. Hamann S, Zeuthen T, La Cour M et al. Aquaporinsin complex tissues distribution of aquaporins 1 – 5in human and rat eye. Am J Physiol 1998; 274: 1332 –
28. Lerche W. Electron microscopic observations ofBruch' s membrane in the human eye. Ber Zusam-menkunftDtschOphthalmolGes 1964; 65: 384 – 5.
29. Bell E. Experimental studies on the development ofthe eye and the nasal cavities in frog embryos. AnatAnz1906; 29: 185 – 94.
30. Jain A, Saxena S, Khanna VK, Shukla RK, Meyer CH. Status of serum VEGF and ICAM-1 and its association with external limiting membrane and inner segment-outer segment junction disruption in type 2 diabetes mellitus. Mol Vis. 2013;19:1760 – 8.
31. Strozecki P, Kurowski R, Flisinski M, Stefanska A, Odrowaz-Sypniewska G, Manitius J. Advanced glycation end products and arterial stiffness in diabetic and non-diabetic patients with chronic kidney disease. Pol Arch Med Wewn. 2013;123:609 – 16.
32. Sharma S, Saxena S, Srivastav K, Shukla R, Mishra N, Meyer C, et al. Nitric oxide levels in diabetic retinopathy and its association with disruption of photoreceptor IS-OS junction and topographic alterations in retinal pigment epithelium. Clin Exp Ophthalmol. 2015;43:429 – 36.
33. Mishra N, Saxena S, Shukla RK, Singh V, Meyer CH, Kruzliak P, et al. Association of serum N ε -carboxy methyl lysine with severity of diabetic retinopathy. J Diab Complic. 2016;30(30):511 – 7.
34. Patz A. Retinal neovascularisation: early contributions of Professor Michaelson and recent observations. Br J Ophthalmol. 1984;68:42 – 6.
35. Crawford TN, Alfaro DV, Kerrison JB, Jablon EP. Diabetic retinopathy and angiogenesis. Curr Diabetes Rev. 2009;5:8 – 13.
36. Funatsu H, Yamashita H, Noma H, Shimizu E, Yamashita T, Hori S. Stimulation and inhibition of angiogenesis in diabetic retinopathy. Jpn J Ophthalmol. 2001;45:577 – 84.
37. Fry DL. Certain histological and chemical responses of the vascular interface to acutely induced mechanical stress in the aorta of the dog. Circ Res. 1969;24:93 – 108.
38. Tooke JE. Microvascular haemodynamics in diabetes mellitus. Clin Sci. 1986;70:119 – 25.
39. Burton AC. Relation of structure to function of the tissues of the walls of blood vessels. Physiol Rev. 1954;34:619 – 42.
40. Grunwald JE, DuPont J, Riva CE. Retinal haemodynamics in patients with early diabetes mellitus. Br J Ophthalmol. 1996;80:327 – 31.
41. Grunwald JE, Riva CE, Martin DB, Quint AR, Epstein PA. Effect of an insulin-induced decrease in blood glucose on the human diabetic retinal circulation. Ophthalmology. 1987;94:1614 – 20.
42. Sandeep Saxena, GauharNadri, Apjit Kaur, Abbas Mahdi, Kaleem Ahmad, Pragati Garg; Low serum vitamin D levels correlate with disorganization of retinal inner layers, ellipsoid zone disruption and retinal pigment epithelium alterations in diabetic retinopathy. Invest. Ophthalmol. Vis. Sci. 2019;60(9):5321.