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Review Article

Pathogenesis of Diabetic Retinopathy: Biochemical Aspects and Therapeutic Approaches

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Abstract: Diabetic retinopathy is one of the reasons for severe loss of visual acuity in diabetic patients. Exposure to hyperglycemic environment is the root cause, which activates or increases the rate of several biochemical pathways such as, polyol pathway, PKC pathway and formation of advanced glycation end products in the tissues leading to hypoxia, followed by tissue damage. Oxygen deprivation stimulates the retina to release growth factors causing growth of undesirable blood vessels in the retina, which is the central cause for retinopathy. Laser and anti-VEGF therapies are being used worldwide as a mode of treatment. But newer approaches to an effective treatment with less side effects is always a matter of concern. So several therapeutic agents to block the basic biochemical pathways of pathogenesis are designed and under research. This review focuses on the basic biochemical pathways for the pathogenesis of retinopathy and the drugs designed to block those pathways for the treatment of diabetic retinopathy.
Keywords: Diabetic Retinopathy, Treatment, polyol pathway, Advanced glycation end products, PKC pathway, Leukostasis.

INTRODUCTION

Diabetic retinopathy (DR) is considered to be the most serious complication and one of the major causes of blindness in diabetic patients. It is a vascular disorder affecting the microvasculature of the retina. Usually, retinopathy was not found to be clinically significant during early years of diagnosis of diabetes. It has been found that 25-50% of the type1 diabetic population showed some degree of retinopathy within 10 to 15 years and 75% within 15 to 20 years of diagnosis of type 2 diabetes mellitus. However 20% of type2 diabetic patients found to have retinopathy by the time they are diagnosed with diabetes [1,2].In addition, several factors such as, duration of diabetes mellitus, hypertension, hyperlipidemia, nephropathy, poor glycemic control, anemia, alcohol consumption and pregnancy can influence the development and progression of diabetic retinopathy [3-6].

Hyperglycemia is a key factor in the development of diabetic retinopathy which can activate multiple biochemical pathways involved in the pathogenesis of diabetic retinopathy. It causes increased flux of glucose through several pathways mainly, hexosamine pathway, aldose reductase and protein kinase C (PKC) pathway. Activation of hexosamine pathway can further causes altered glycosylation of transcription factors and activation of inflammatory genes [7]. Non enzymatic glycosylation can leads to functional alteration of several proteins. Increased formation of sugar alcohols (such as sorbitol) from aldose reductase pathway can deplete NADPH required for the cell to fight against oxidative stress [8]**.** Protein kinase C pathway activation can trigger changes in retinal blood flow, thickening of the basement membrane, extracellular matrix expansion and increase in vascular permeability [7]. All these factors finally cause endothelial cell dysfunction, pericyte apoptosis, microvascular leakage and microaneurysms leading to retinal ischemia which can trigger angiogenesis in retina which is a hallmark of diabetic retinopathy.

Microvascular leakage caused by increased expression of potent vasoactive molecules such as Vascular Endothelial Growth Factor (VEGF), Transforming Growth Factor-β (TGF-β), Interleukin-1 (IL1), Tumor Necrosis Factor-(TNF-α), Matrix Metalloproteinases (MMPs), etc. can result in development of macular edema (ME). The same vasoactive molecules can also trigger hypoxia induced neovascularization in the retina [9-13].The new blood vessels thus formed are thin and fragile. They can

rupture and bleed into the vitreous cavity leading to loss of visual acuity, contraction of the vitreous, retinal detachment and severe loss of vision [14-17].

Laser photocoagulation therapy has been used worldwide as the most effective course of treatment in preventing severe vision loss in diabetic patients [18,19]. Anti-VEGF agents are also in use to trap VEGF and to stop macular edema as well as angiogenesis [20,21]. In recent years, several anti-angiogenic molecules such as PKC inhibitors, tyrosine kinase receptor blockers etc. have been tried and are under clinical trial for the treatment of angiogenesis in diabetic retinopathy.

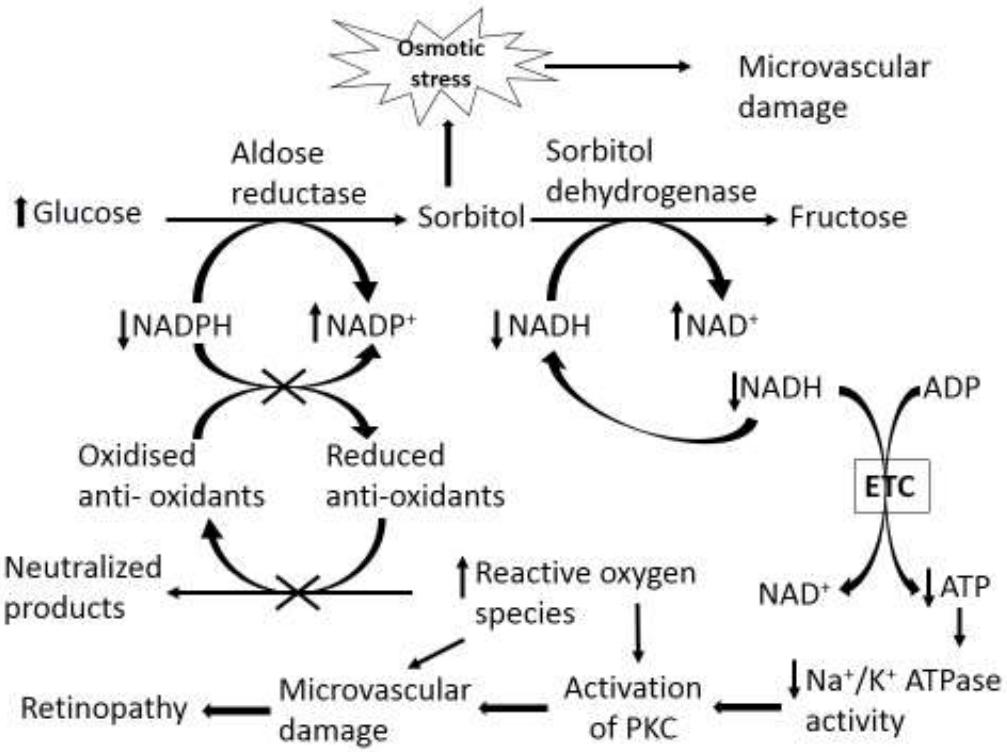
Pathogenesis of diabetic retinopathy a biochemical view:

One of the most important factors in the pathogenesis of diabetic vascular complications is hyperglycemia induced abnormal activation of several metabolic pathways. A major part of it comprises of hyperactive polyol pathway, increased flux of glucose through hexosamine pathway, oxidative stress and increased activation of PKC pathway.

Polyol pathway

Cells or tissues in the hyperglycemic milieu which are independent of insulin [nerve fibers, retina, lens, kidney etc.] can freely uptake glucose, which will be acted upon by aldose reductase that reduces glucose to sorbitol and $NADP^+$. Further sorbitol dehydrogenase oxidizes sorbitol to fructose and NAD^+.This reaction consume NADPH and $NADP^+$ starts accumulating with compromising anti-oxidant defense system. The excessively produced sorbitol simultaneously starts accumulating in these tissues also cause osmotic stress[22-26]. Aldose reductase (AR) is the rate limiting enzyme in polyol pathway. Expression of aldose reductase protein have been shown to be elevated in nerve fibers, ganglion cells and Muller cells derived from diabetic patients with retinopathy when compared to no diabetic individuals [7,26,27]. Rate of polyol formation in *ex vivo* rat retinas have shown increased flux of glucose through polyol pathway which increase with the duration of diabetes and hyperglycemia [28]. Julia V. Busik, *et al.* 2002 showed that hyperglycemia itself causes increased uptake of glucose by retinal endothelial cells and pigment epithelial cells in culture, coupled with accumulation of sorbitol in tissues [29].

In addition to decrease cytosolic NADPH, polyol pathway also found to increase the ratio of $NADH/NAD^+$. The remarkable detrimental effects of this pathway also include, decrease in the activity of Na^+/K^+ ATPase enzyme, activation of PKC pathway, a compromise in the antioxidant defense, which collectively can cause micro vascular damage, leading to diabetic retinopathy and other complications of diabetes [8,30,31][Fig. 1].

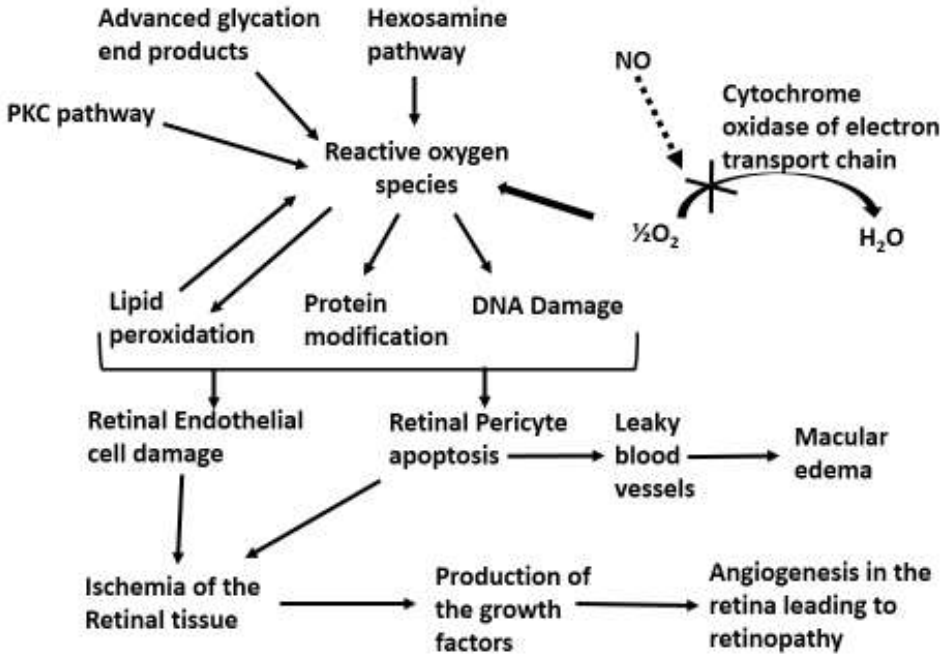


(↑= increase; ↓= decrease; X= blocked, ETC- Electron Transport Chain)

Fig-1: Showing Polyol pathway and its effects

Oxidative stress

Another pathophysiological condition in diabetes is an increase in the oxidative stress. When compare to other tissues retina has a high oxygen uptake and lipid peroxidation as its polyunsaturated fatty acids content is high. This is the reason why retina is more susceptible to oxidative stress [32].Several studies have indicated that, reactive oxygen species (ROS) activate signaling pathways that promote angiogenesis [33-35]. Vascular endothelial cells and smooth muscle cells contain NAD(P)H oxidases and are found to be good source of ROS formation from molecular oxygen [36,37]. NO is a vasodilator, synthesis of which is upregulated in diabetes. Nitric Oxide has been shown to promote ROS production. Possible mechanism could be interference of nitric oxide (NO) in reaction of mitochondrial cytochrome c oxidase enzyme complex preventing oxygen reduction to water, thereby promoting ROS production by the reactive oxygen molecule [38-39].



(**X** = blocked, ▪▪▪ = Inhibition)

Fig-2: Showing Oxidative stress; sources and consequences

Hyperactive aldose reductase, PKC pathway, hexosamine pathway, variation in the metabolism of lipoproteins, advanced glycated end products (AGE), induced by hyperglycemia could be the major sources for ROS [40].Structural changes in the biomolecules (DNA and protein modification and lipid per oxidation) due to ROS attack decide the functional status and thereby integrity of all the cellular reactions. Among all the body cells pericytes were found to be highly responsive to oxidative stress which was proven by several *in vivo* studies where lack of their function or loss of pericytes itself have been shown to augment endothelial proliferation [28, 41-46]. When the loss crosses a particular limit blood vessels become fragile and start bleeding leading to macular edema. This may end up in development of diabetic retinopathy [Fig. 2].

Non-enzymatic glycation reactions and formation of advanced glycated end products

Advanced glycated end products are formed by the Maillard reaction. It is a non-enzymatic reaction between aldehyde group of a glucose molecule and amino group of a protein. Advanced glycated end products have been implicated in the aging of proteins and alteration in their function [49,50].These complex molecules induce specific effects by binding to specific receptors on the target tissue called receptors for advanced glycated end products (RAGEs). There were two types of receptors found. One that is anchored on cell membrane and another freely circulating form lacking transmembrane domain. Membrane bound form of RAGE are involved in pathogenic effects of AGEs. In contrast the soluble extracellular ligand binding domains formed by alternative splicing of RAGE mRNA, scavenge AGEs and prevent damages caused by them [51-53].

In pericytes AGEs found to induce apoptosis with an increased activity of caspase-3, caused due to a decreased Bcl-2/Bax ratio and nuclear factor-κB (NF-κB) activation [54,55]. Since pericytes play an important role in the maintenance of micro vascular homeostasis, loss of pericytes can induce, endothelial cell (EC) injury in the retinal blood vessels and angiogenesis, leading to diabetic retinopathy [Fig. 3] [48].

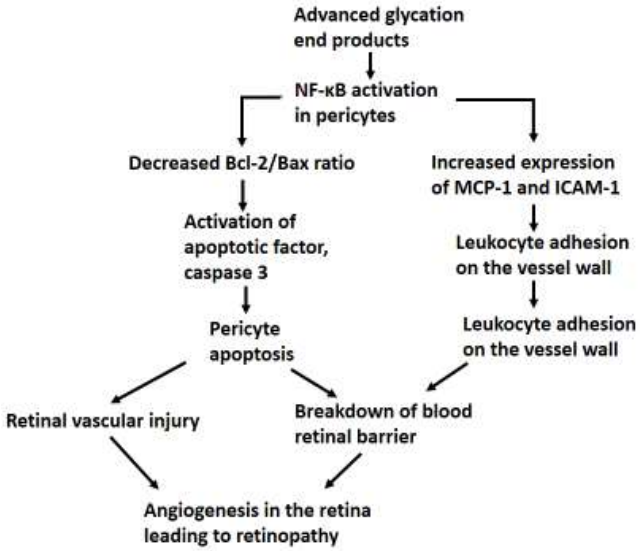


Fig-3: Showing Advanced glycation end product induced angiogenesis in the retina

Literature show, through the intracellular ROS generation AGEs can induce the expression of intracellular cell adhesion molecule-1 (ICAM-1) and monocyte chemo attractant protein-1 (MCP-1) by micro vascular ECs. These events can induce leukocyte adhesion on ECs, leading to breakdown of blood retinal barrier (BRB) (consisting of retinal vascular endothelium and retinal pigment epithelium which maintain the transport of substances between blood and retinal tissue) [7,53,56]. Ming Lu, 1997 in an *in vitro* study found that AGE-induces VEGF expression in a dose and time-dependent manner. Anti-VEGF antibody has shown to block capillary endothelial cell proliferation in these tissues exposed to AGE [57]. As AGEs have the ability to increase retinal VEGF gene expression they might be involved in the pathogenesis of diabetic retinopathy.

Hexosamine pathway was proposed to be one of the culprits for insulin resistance, growth factors expression and there bydiabetic vascular complications. This pathway involves the conversion of fructose-6-phosphate to glucosamine-6-phosphate by glutamine: fructose -6-phosphate amidotransferase (GFAT).This is a rate-limiting step in this pathway. Glucosamine-6-phosphate will be rapidly metabolized to UDP-N-acetyl-glucosamine. Hexosamines found to be involved in the synthesis of glycoproteins, gangliosides, proteoglycans, glycolipids etc.[58-61]. Altered glycation of the transcription factors found to alter the expression of inflammatory genes. Studies regarding the role of GFAT in ophthalmic complications of diabetes are lacking. But it is hypothesized that undue channeling of glucose via hexosamine pathway might affect neuroprotective effect of insulin thereby inducing apoptosis of retinal neurons.

Hexosamine pathway

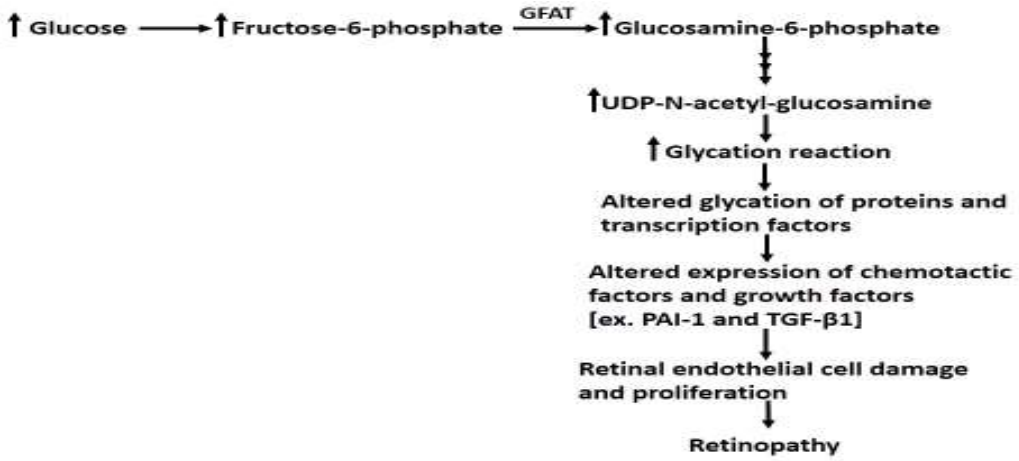


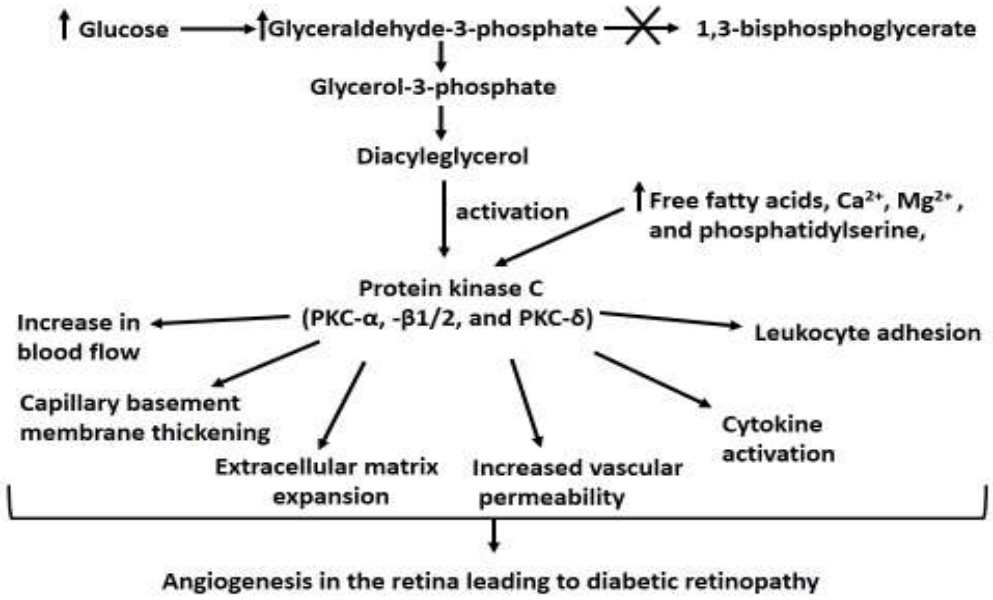
Fig-4: Showing Association of hexosamine pathway with diabetic retinopathy

This could be mediated by protein kinase B and possibly linked with altered glycosylation of proteins [62,63]. Reports have shown involvement of hexosamine pathway in the expression of transforming

growth factor-β1 (TGF-β1), plasminogen activator inhibitor-1 (PAI-1) etc. which are already proved to be the culprits for several vascular complications in the diabetes [7,64] [Fig 4].

Increased PKC activation

The protein kinase C is a large group of structurally related family of serine/threonine kinases involved in cell signaling cascade that mediates several unique functions. The family of PKCs includes at least eleven isoforms (α, β1, β2 etc.) and are the key targets for lipid second messengers in the downstream signaling cascade [65,66]. Ca^{2+} ions, diacylglyceral (DAG), phosphatidylserine are found to be some of the activators of these molecules. Increased *de novo* DAG synthesis has been seen in animal models due to the inhibition of glyceraldehyde-3-phosphate dehydrogenase or accelerated reduction of dihydroxyacetone phosphate to glycerol-3-phosphate. Researchers stated that it would even activate PKC in cultured vascular cells, retina and glomeruli *in vivo*[62,67]. The activities of PKC-α, -β1, -β 2, and -δ were found to be stimulated immensely with DAG and found to be linked with pathogenesis of diabetic retinopathy [58]. PKC activation can cause vasoconstriction in the retina by increasing the expression of endothelin-1 (ET-1) [68]. Consequences of PKC activation would be an increase in blood flow through capillaries, increased vascular permeability, cytokine activation, basement membrane thickening, angiogenesis, which ends with retinopathy and other complications tangled with diabetes [7,68-70] [Fig 5].



(↑= Increased; **X** = Blocked)

Fig-5: Showing PKC activation in the pathogenesis of diabetic retinopathy

Leukostasis

Aggregation of leukocytes on the vessel wall was found to be one of the causes and earlier events in the pathogenesis of diabetic retinopathy. Leukocytes have a capacity to adhere to the vascular endothelium, and can produce highly toxic superoxide radicals and proteolytic enzymes [71]. Adhesion process was shown to be mediated by a member of immunoglobulin supergene family of cellular adhesion molecules called, vascular cell adhesion molecule (VCAM) which acts as a chemotactic factor for leukocytes, help in adhesion to vascular wall, and if required migrating to adjacent tissue [72].

Vascular endothelial growth factor is shown to promote leukostasis and vascular leakage, through the activation of intracellular cell adhesion molecule-1 (ICAM-1) in the diabetic retinas [73]. In addition, AGEs were also been demonstrated to increase leukostasis by increasing the expression of ICAM-1 in retinal microvascular endothelial cells in culture [53,74].

Blockages in capillaries and formation of acellular capillaries found to be associated with leukostasis in the diabetic retina [75]. Blockage of ICAM-1 by monoclonal antibodies prevented leukocyte adhesion and found to maintain the BRB, which would indicate one of the primary events in diabetic retinopathy is ICAM-1upregulation [76]. Also, inhibition of the AGE receptor (RAGE) with Pigment Epithelial Derived Factor (PEDF) or pyridoxal phosphate had been shown to inhibit leukostasis and expression of ICAM [77]. So inhibition of leukostasis may prevent endothelial cell and pericyte death which may prevent diabetic retinopathy.

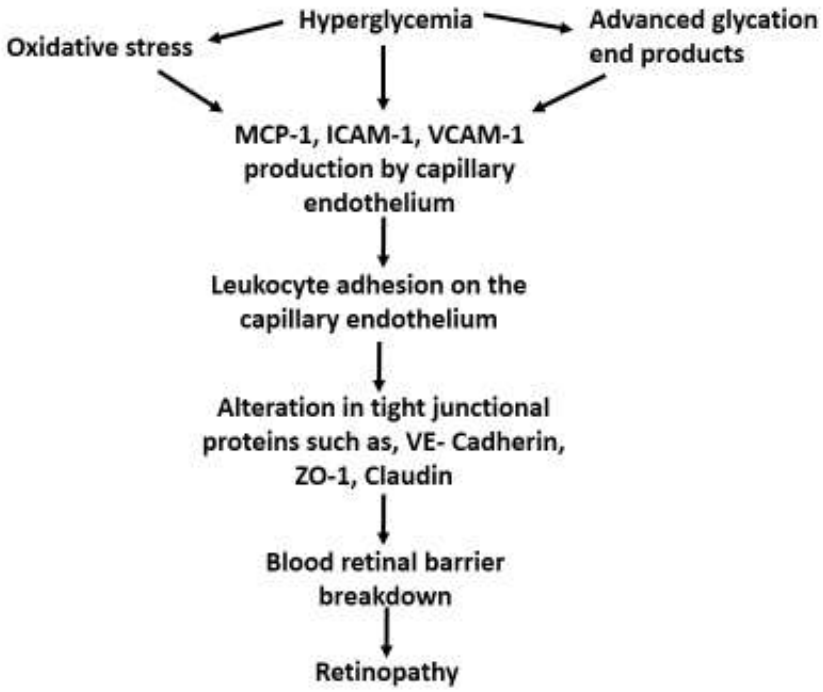


Fig-6: Showing Involvement of leukostasis in the pathogenesis of retinopathy

It is found that, expression of MCP-1 in vascular endothelium is increased due to hyperglycemia. AGEs were also found to trigger the expression MCP-1 gene by inducing ROS generation [72].Increased numbers of leukocytes, in patients with retinopathy found to be significantly correlated with an increased expression of retinal ICAM-1 and CD18 in retinal endothelium. The CD18 molecules on the leukocytes helps leukocytes to attach themselves to intercellular adhesion molecule-1 (ICAM-1) on the surface of retinal endothelium in diabetic animals [78].

The increased leukostasis and recruitment of macrophages and other inflammatory cells mediated by over expression of cell adhesion molecules shown to cause alteration of tight jounctional proteins such as, VE- Cadherin, ZO-1, Claudin. This causes infiltration of inflammatory cells into the retinal tissue, leading to breakdown of the blood retinal barrier which is a hallmark of diabetic retinopathy [Fig 6] [79].

THERAPEUTIC APPROACHES

Inhibitors of Polyol pathway

Trials carried out by inhibiting aldose reductase yielded inconsistent results. The long-term sorbinil (an inhibitor of aldose reductase) trial also indicated that sorbinil found to reduce the rate of progression of microaneurysms, but failed to check the worsening of retinopathy [80]. Trials with zenarestat (aldose reductase inhibitor), gave hopeful results with diabetic neuropathy, which need to be validated for future use for retinopathy [62].Six months double blind study with tolrestat was failed to show any clinically significant cure for retinopathy [81].The trial carried out by Dagher Z, 2004 showed that apoptosis of pericytes and endothelial cells, activation of complement system in the capillary wall thereby formation of acellular capillaries can be prevented by the use of aldose reductase inhibitors [24].

PKC inhibitors

PKC inhibitors were well studied and showed certain hope in treatment of diabetic retinopathy. This was supported by several studies in which, leukostasis, endothelial cell proliferation, angiogenesis, and permeability induced by VEGF were suppressed by the use of PKC- β selective inhibitor ruboxistaurin or LY333531 [82-84]. New phase III clinical trial (Protein Kinase C-β Inhibitor–Diabetic Retinopathy Study) with ruboxistaurin reported decrease in the progression of retinopathy, reduced requirement for Laser photocoagulation and improved visual acuity in diabetic retinopathy patients [85].Ruboxistaurin also found to act as an anti-angiogenic agent via suppression of phosphorylation of ERK1/2 and Akt [86]. Treatment of human retinal microvascular endothelial cells with Rottlerin, transfection of PKC-δ-DN, or siRNA for PKC-δ all found to decrease the vascular leakage and restored lost tight junctional proteins, ZO-1 and ZO-2. Which showed that inhibition of PKC-δ can prevent breakdown of blood retinla barrier [87]. This indicates PKC inhibitors could be used for the treatment of diabetic retinopathy.

Advanced glycated end product inhibitors

Inhibitors of Advanced glycated end product formation had also shown a promising results in improvement of diabetic retinopathy. A recently found inhibitor LR-90 had shown to reduce the death of reinal capillary pericytes and endothelial cells by preventing

AGE formation, thereby reducing the formation of acellular capillaries in streptozotocine induced diabetic rats [88,89]. A RAGE-Ig fusion protein in diabetic rats shown to inhibit capillary degeneration, accumulation of albumin in the neural retina, nitration of retinal proteins, retinal leukostasis and ICAM-1 expression in streptozotocine induced diabetic rats [90]. The systemic administration of sRAGE in diabetic rats found to significantly inhibit blood–retinal barrier breakdown, leukostasis, and expression of ICAM-1 in the retina [91].Olmesartan, angiotensin II type 1 receptor blocker shown to inhibit the AGE-induced NF-kappaB promoter activity and expression of RAGE gene in cultured micro vascular endothelial cells. It was also shown to block AGE-induced up-regulation of VEGF mRNA levels and further increase in DNA replication in the endothelial cells [92].Sulforaphane and RAGE-Ab shown to inhibit the AGE-induced decrease in DNA synthesis, apoptotic cell death, and up-regulation of monocyte chemo attractant protein-1 mRNA levels in pericytes [93].

Antioxidants

As a therapeutic strategy to specifically target ROS in patients with PDR, Yamagishi S, et. al. 2011 proposed that, PEDF could be a best treatment option for retinopathy as it has an anti-oxidative, anti-angiogenic, anti-inflammatory and neuroprotective effects [94]. Vitamin C and E supplementation in type2 diabetic retinopathy patients found to reduce NO induced stress in the eyes [95].Pazdro R, *et al.* demonstrated a protective role of Vitamin E against lipid per oxidation, however its effects on protein and DNA oxidation is yet to be evaluated [96].Treatment of diabetic rats with retinopathy, with lipoic acid had demonstrated a reduced pericyte loss and formation of acellular capillaries in the retinal capillary endothelial cells. This had also shown to reduce the expression of angiogenic factors, angiopoietin-2 and VEGF [97]. An intracellular labile iron chelator, salicylaldehydeisonicotinoyl hydrazine has been shown to reduce the apoptosis of retinal pigment epithelial cells induced by hydrogen peroxide *in vitro* have been proven to be use full in several disease conditions [98].So anti-oxidants have been found to be useful for the management of retinopathy. However appropriate dose and combination of antioxidants, better root for the administration and their side effects are yet to be addressed.

REFERENCES

1. Fong DS, Aiello L, Gardner TW, King GL, Blankenship G, Cavallerano JD, *et al.*; Diabetic Retinopathy. Diabetes Care, 2003; 26(1):s99-s102.
2. Fong DS, Aiello L, Gardner TW, King GL, Blankenship G, Cavallerano JD, *et al.*; Retinopathy in diabetes. Diabetes Care, 2004; 27(1):S84-87.
3. Rema M, Pradeepa R; Diabetic retinopathy: An Indian perspective. Indian Journal of Medical Research, 2007;125: 297-310.
4. Senthilvel V, Radhakrishnan R, Sathiyamoorthi R, Sumathi S, Jayanthi V; A study on finding influencing factors on diabetic retinopathy among diabetic patients using Multiple Regression approach. Journal of Dental and Medical Sciences, 2012; 1(4):20-23.
5. Suzuma I, Hata Y, Clermont A, Pokras F, Rook S. L, Suzuma K, *et al.;* Cyclic stretch and hypertension induce retinal expression of vascular endothelial growth factor and vascular endothelial growth factor receptor–2. Diabetes, 2001; 50(2):444-454.
6. Rassam SM, Patel V, Kohner M; The effect of experimental hypertension on retinal vascular autoregulation in humans: a mechanism for the progression of diabetic retinopathy. Experimental Physiology, 1995; 80(1):53-68.
7. Cellular and Molecular Mechanism of Diabetic Retinopathy. Available from http://cdn.intechopen.com/pdfs-wm/29184.pdf.
8. Obrosova IG, Stevens MJ, Lang HJ; Diabetes-induced changes in retinal NAD-redox status. Pharmacology, 2001; 62(3):172-180.
9. Biomarkers in diabetic retinopathy and the therapeutic implications. Available from http://www.hindawi.com/journals/mi/2013/193604/ .
10. Funatsu H, Yamashita H, Noma H, Mimura T, Yamashita T, Hori S; Increased levels of vascular endothelial growth fact or and interleukin-6 in the aqueous humor of diabetics with macular edema. American Journal of Ophthalmology, 2002;133(1):70-77.
11. Funatsu H, Yamashita H, Noma H, Mimura T, Nakamura S, Sakata K; Aqueous humor levels of cytokines are related to vitreous levels and progression of diabetic retinopathy in diabetic patients. Graefe's Archive for Clinical and Experimental Ophthalmology, 2005;243(1):3-8.
12. Navaratna D, McGuire PG, Menicucci G, Das A; Proteolytic degradation of VE-cadherin alters the blood-retinal barrier in diabetes. Diabetes, 2007; 56(9): 2380-2387.
13. Hollborn M, Stathopoulos C, Steffen A, Wiedemann P, Kohen L, Bringmann A; Positive feedback regulation between MMP-9 and VEGF in human RPE cells. Investigative Ophthalmology & Visual Science, 2007; 48(9):4360-4367.
14. Bhagat N, Grigorian RA, Tutela A, Zarbin MA; Diabetic macular edema: pathogenesis and treatment. Survey of ophthalmology, 2009; 54(1):1-32.
15. Spraul CW, Grossniklaus HE; Vitreous hemorrhage. Survey of ophthalmology, 1997;42(1):3-39.

16. Johnson, Mark W; Etiology and treatment of macular edema. American journal of ophthalmology, 2009; 147(1):11-21.
17. Zhang W, Liu H, Rojas M, Caldwell RW, Caldwell RB; Anti-inflammatory therapy for diabetic retinopathy. Immunotherapy, 2011; 3(5):609-628.
18. Praidou A, Androudi S, Brazitikos P, Karakiulakis G, Papakonstantinou E, Tsinopoulos I, et al.; Diabetic retinopathy treated with laser photocoagulation and the indirect effect on glycaemic control. Journal of diabetes research, 2014:1-3.
19. Graham CE, Binz N, Shen WY, Constable IJ, Rakoczy EP; Laser Photocoagulation: Ocular Research and Therapy in Diabetic Retinopathy. Advances in Experimental Medicine and Biology, 2006; 572:195-200.
20. Jardeleza MSR, Miller JW; Review of anti-VEGF therapy in proliferative diabetic retinopathy. Seminars in ophthalmology, 2009; 24(2):87-92.
21. Waisbourd M, Goldstein M Loewenstein A; Treatment of diabetic retinopathy with anti-VEGF drugs. Actaophthalmologica, 2011; 89(3):203-207.
22. Chung SS, Ho EC, Lam KS, Chung SK; Contribution of polyol pathway to diabetes-induced oxidative stress. Journal of the American Society of Nephrology, 2003; 14(3):S233-S236.
23. Lee AY, Chung SS; Contributions of polyol pathway to oxidative stress in diabetic cataract. The FASEB Journal, 1999; 13(1):23-30.
24. Dagher Z, Park YS, Asnaghi V, Hoehn T, Gerhardinger C, Lorenzi M; Studies of rat and human retinas predict a role for the polyol pathway in human diabetic retinopathy. Diabetes, 2004; 53(9):2404-2411.
25. Brownlee, Michael; the pathobiology of diabetic complications a unifying mechanism. Diabetes, 2005; 54(6):1615-1625.
26. Figueroa-Romero C, Sadidi M, Feldman EL; Mechanisms of disease: the oxidative stress theory of diabetic neuropathy. Reviews in Endocrine and Metabolic Disorders, 2008; 9(4): 301-314 .
27. Abu El-Asrar AM, Al-Mezaine HS, Ola MS; Pathophysiology and management of diabetic retinopathy. 2009;4: 627-647.
28. Ola MS, Berkich DA, Xu Y, King MT, Gardner TW, Simpson I, *et al.*; Analysis of glucose metabolism in diabetic rat retinas. American Journal of Physiology - Endocrinology and Metabolism, 2006:290(6):E1057-E1067.
29. Busik, JV, Olson LK, Grant MB, Henry DN; Glucose-induced activation of glucose uptake in cells from the inner and outer blood-retinal barrier. Investigative Ophthalmology and Visual Science, 2002; 43(7):2356-2363.
30. Van den Enden MK, Nyengaard JR, Ostrow E, Burgan JH, Williamson JR; Elevated glucose levels increase retinal glycolysis and sorbitol pathway metabolism. Implications for diabetic retinopathy. Investigative ophthalmology & visual science, 1995; 36(8): 1675-1685
31. Ido Y, Nyengaard JR, Chang K, Tilton RG, Kilo C, Mylari BL; Early neural and vascular dysfunctions in diabetic rats are largely sequelae of increased sorbitol oxidation. Antioxidants & redox signaling, 2010; 12(1):39-51.
32. Oxidativestressanddiabetic retinopathy. Available from http://www.hindawi.com/journals/jdr/2007/043603/abs/.
33. Raquel Soares; Angiogenesis in Diabetes. Unraveling the Angiogenic Paradox. The Open Circulation and Vascular Journal, 2010; 3:3-9.
34. Thannickal VJ, Fanburg BL; Reactive oxygen species in cell signaling. American Journal of Physiology - Lung Cellular and Molecular Physiology, 2000; 279: L1005-L1028.
35. Reactiveoxygenspeciesasmediatorsofangiogenesissignaling.Availablefrom http://www.pharmacy.upatras.gr/index.php/en/latest-news/doc_download/2049-ergasia2.
36. Brandes RP, Kreuzer J; Vascular NADPH oxidases: molecular mechanisms of activation. Cardiovascular research, 2005; 65(1):16-27.
37. Patterson C, Ruef J, Madamanchi NR, Barry-Lane P, Hu Z, Horaist C, *et al.*; stimulation of a vascular smooth muscle cell NAD(P)H oxidase by thrombin evidence that p47 phox may participate in forming this oxidase in vitro and in vivo. Journal of Biological Chemistry, 1999; 274(28):19814-19822.
38. Palacios-Callender M, Hollis V, Mitchison M, Frakich N, Unitt D, Moncada S; Cytochrome c oxidase regulates endogenous nitric oxide availability in respiring cells: a possible explanation for hypoxic vasodilation. Proceedings of the National Academy of Sciences of the United States of America, 2007;104:18508-18513.
39. Zhang DX, Gutterman DD; mitochondrial reactive oxygen species-mediated signalling in endothelial cells. American Journal of Physiology-Heart and Circulatory Physiology, 2007; 292(5): H2023-H2031.
40. Giusti, C, Gargiulo, P; Advances in biochemical mechanisms of diabetic retinopathy. European Review for Medical and Pharmacological Sciences, 2007; 11: 155-163.
41. Cacicedo JM, Benjachareowong S, Chou E, Ruderman NB, Ido Y; Palmitate-induced apoptosis in cultured bovine retinal pericytes roles of NAD (P) H oxidase, oxidant stress, and ceramide. Diabetes, 2005; 54(6):1838-1845.
42. Amano S, Yamagishi SI, Inagaki Y, Nakamura K, Takeuchi M, Inoue H, *et al.*; Pigment epithelium-derived factor inhibits oxidative stress-induced apoptosis and dysfunction of cultured retinal pericytes. Microvascular research, 2005; 69(1):45-55.

43. Li W, Yanoff M, Jian B, He Z; Altered mRNA levels of antioxidant enzymes in pre-apoptotic pericytes from human diabetic retinas. Cellular and molecular biology (Noisy-le-Grand, France), 1999;45(1):59-66.
44. Gerhardt H, Betsholtz C; Endothelial-pericyte interactions in angiogenesis. Cell and tissue research, 2003; 314(1):5-23.
45. Hammes HP, Lin J, Renner O, Shani M, Lundqvist A, Betsholtz C, Deutsch U; Pericytes and the pathogenesis of diabetic retinopathy. Diabetes, 2002; 51(10):3107-3112.
46. Ejaz S, Chekarova I, Ejaz A, Sohail A, Lim CW; Importance of pericytes and mechanisms of pericyte loss during diabetic retinopathy. Diabetes, Obesity and Metabolism, 2008; 10(1):53-63.
47. Vinores SA, Derevjanik NL, Ozaki H, Okamoto N, Campochiaro PA; Cellular mechanisms of blood-retinal barrier dysfunction in macular edema. DocumentaOphthalmologica, 1999; 97(3-4):217-228.
48. Yamagishi SI, Ueda S, Matsui T, Nakamura K, Okuda S; Role of advanced glycation end products (AGES) and oxidative stress in diabetic retinopathy. Current Pharmaceutical Design, 2008:14:962-968.
49. Jakuš V, Rietbrock N; Advanced glycation end-products and the progress of diabetic vascular complications, Physiological Research, 2004:53:131-142.
50. Lu M, Kuroki M, Amano S, Tolentino M, Keough K, Kim I; Advanced glycation end products increase retinal vascular endothelial growth factor expression. The American Society for Clinical Investigation, 1998; 101(6): 1219–1224.
51. Goldin A, Beckman JA, Schmidt AM, Creager MA; Advanced glycation end products: sparking the development of diabetic vascular injury. Circulation, 2006; 114: 597-605.
52. Yamagishi SI, Matsui T; Advanced glycation end products (AGEs), oxidative stress and diabetic retinopathy. Current pharmaceutical biotechnology, 2011; 12(3):362-368.
53. Gaonkar B, Prabhu K; Advanced Glycation End Products in the Pathogenesis of Diabetic Retinopathy: a Review. Asian Journal of Pharmaceutical Research, 2015;5(2):134-142.
54. Yamagishi S, Inagaki Y, Amano S, Okamoto T, Takeuchi M, Makita Z; Pigment epithelium-derived factor protects cultured retinal pericytes from advanced glycation end product-induced injury through its antioxidative properties. Biochemical and Biophysical Research Communications, 2002; 296: 877-82.
55. Amano S, Yamagishi S, Inagaki Y, Nakamura K, Takeuchi M, Inoue H, *et al.*; Pigment epithelium-derived factor inhibits oxidative stress-induced apoptosis and dysfunction of cultured retinal pericytes. Microvascular Research, 2005; 69: 45-5.
56. Inagaki Y, Yamagishi S, Okamoto T, Takeuchi M, Amano S; Pigment epithelium-derived factor prevents advanced glycation end products-induced monocyte chemoattractant protein-1 production in microvascular endothelial cells by suppressing intracellular reactive oxygen species generation. Diabetologia, 2003; 46: 284-7.
57. Lu M, Kuroki M, Amano S, Tolentino M, Keough K, Kim I *et al.*; Advanced glycation end products increase retinal vascular endothelial growth factor expression. The American Society for Clinical Investigation, 1998; 101: 1219–1224.
58. Balasubramanyam M, Rema M, Mohan V, Premanand C; Biochemical and molecular mechanisms of diabetic retinopathy. Current Science, 2002; 83(12):1506-1514.
59. Nerlich AG, Sauer U, Kolm-Litty V, Wagner E, Koch M, Schleicher ED; Expression of glutamine: fructose-6-phosphate amidotransferase in human tissues: evidence for high variability and distinct regulation in diabetes. Diabetes, 1998; 47: 170–178.
60. Wells L, Vosseller K, Hart GW; A role for N-acetylglucosamine as a nutrient sensor and mediator of insulin resistance. Cellular and Molecular Life Sciences, 2003; 60(2):222-228.
61. Masson E, Wiernsperger N, Lagarde M, El Bawab S; Involvement of gangliosides in glucosamine-induced proliferation decrease of retinal pericytes. Glycobiology, 2005; 15(6) :585-591.
62. Molecular mechanisms of diabetic retinopathy, general preventive strategies, and novel therapeutic targets. Available from http://www.hindawi.com/journals/bmri/2014/801269/cta/.
63. Nakamura M, Barber AJ, Antonetti DA, LaNoue KF, Robinson KA, Buse MG *et al.*; Excessive hexosamines block the neuroprotective effect of insulin and induce apoptosis in retinal neurons. The Journal of Biological Chemistry, 2001; 276: 43748–43755.
64. Buse MG; Hexosamines, insulin resistance, and the complications of diabetes: current status. American Journal of Physiology-Endocrinology And Metabolism, 2006; 290(1): E1-E8.
65. Koya D, King GL; Protein Kinase C activation and the development of diabetic complications. Diabetes, 1998;47: 859–866.
66. Nishizuka Y; Protein kinase C and lipid signaling for sustained cellular responses. FASEB, 1995; 9:484–496.
67. Koya D, King GL; Protein kinase C activation and the development of diabetic complications, Diabetes, 1998; 47:859–866.
68. Geraldes P, Hiraoka-Yamamoto J, Matsumoto M, Clermont A, Leitges M, Marette A, *et al.*; Activation of PKC-delta and SHP-1 by hyperglycemia causes vascular cell apoptosis and diabetic retinopathy. Nature Medicine, 2009;15:1298–1306.

69. Aiello LP, Davis MD, Girach A, Kles KA, Milton RC, Sheetz MJ, *et al.*; Effect of ruboxistaurin on visual loss in patients with diabetic retinopathy. Ophthalmology, 2006; 113(12):2221-2230.
70. Evcimen ND, King G L; The role of protein kinase C activation and the vascular complications of diabetes. Pharmacological Research, 2007; 55(6):498-510.
71. Miyamoto K, Ogura Y; Pathogenetic potential of leukocytes in diabetic retinopathy. Seminars in ophthalmology, 1999; 14(4):233-239.
72. Inflammation and Angiogenesis in Diabetic Retinopathy. Available from http://cdn.intechopen.com/pdfs-wm/29192.pdf.
73. Scholl S, Kirchhof J, Augustin AJ; Pathophysiology of macular edema. Ophthalmologica, 2010;224(1):8–15.
74. Moore TC, Moore JE, Kaji Y, Frizzell N, Usui T, Poulaki V, *et al.*; The role of advanced glycation end products in retinal microvascular Leukostasis. Investigative Ophthalmology & Visual Science, 2003; 44: 4457-4464.
75. Ciulla TA, Amador AG, Zinman B; Diabetic retinopathy and diabetic macular edema pathophysiology, screening, and novel therapies. Diabetes care, 2003; 26(9): 2653-2664.
76. Miyamoto K, Khosrof S, Bursell SE, Rohan R, Murata T, Clermont AC,*et al.*; Prevention of leukostasis and vascular leakage in streptozotocin-induced diabetic retinopathy via intercellular adhesion molecule-1inhibition.Proceedings of the National Academy of Sciences USA, 1999; 96:10836-10841.
77. Yamagishi S, Nakamura K, Matsui T, Ueda S, Fukami K, Okuda S; Agents that block advanced glycation end products (AGE)-RAGE (receptor for AGEs)-oxidative stress system: a novel therapeutic strategy for diabetic vascular complications. Expert Opinion on Investigational Drugs, 2008; 17:983-96.
78. El-Asrar AMA, Nawaz MI, Kangave D, Geboes K, Ola MS, Ahmad S, *et al.*; High-mobility group box-1 and biomarkers of inflammation in the vitreous from patients with proliferative diabetic retinopathy. Molecular Vision, 2011; 17:1829–1838.
79. Rangasamy S, McGuire PG, Das A *et al.*; Diabetic retinopathy and inflammation: novel therapeutic targets. Middle East African journal of ophthalmology, 2012; 19(1):52–59.
80. Sorbinil Retinopathy Trial Research Group. A randomized trial of sorbinil, an aldose reductase inhibitor, in diabetic retinopathy. Archives of Ophthalmology, 1990; 108(9):1234-1244.
81. Van Gerven JM, Boot JP, Lemkes HH, Van Best JA; Effects of aldose reductase inhibition with tolrestat on diabetic retinopathy in a six months double blind trial. Documentaophthalmologica, 1994;87(4):355-365.
82. Aiello LP, Bursell SE, Clermont A, Duh E, Ishii H, Takagi C, *et al.*; Vascular endothelial growth factor–induced retinal permeability is mediated by protein kinase C in vivo and suppressed by an orally effective β-isoform–selective inhibitor. Diabetes, 1997; 46(9):1473-1480.
83. Nonaka A, Kiryu J, Tsujikawa A, Yamashiro K, Ogura Y; PKC-beta inhibitor (LY333531) attenuates leukocyte entrapment in retinal microcirculation of diabetic rats. Investigative Ophthalmology & Visual Science, 1999; 40(4):S680-S680.
84. Gardner TW, Antonetti DA; Ruboxistaurin for diabetic retinopathy. Ophthalmology, 2006; 113(12): 2135-2136.
85. Group PD; Effect of ruboxistaurin on visual loss in patients with diabetic retinopathy. Ophthalmology, 2006; 113(12):2221-2230.
86. Nakamura S, Chikaraishi Y, Tsuruma K, Shimazawa M, Hara H; Ruboxistaurin, a PKCβ inhibitor, inhibits retinal neovascularization via suppression of phosphorylation of ERK1/2 and Akt. Experimental eye research, 2010; 90(1):137-145.
87. Kim JH, Kim JH, Jun HO, Yu YS, Kim KW; Inhibition of protein kinase C δ attenuates blood-retinal barrier breakdown in diabetic retinopathy. The American journal of pathology, 2010; 176(3):1517-1524.
88. Bhatwadekar A, Glenn JV, Figarola JL, Scott S, Gardiner TA, Rahbar S, *et al.*; A new advanced glycation inhibitor, LR-90, prevents experimental diabetic retinopathy in rats. British Journal of Ophthalmology, 2008; 92(4):545-547.
89. Figarola JL, Singhal J, Rahbar S, Awasthi S, Singhal SS, *et al.;* LR-90 prevents methylglyoxal-induced oxidative stress and apoptosis in human endothelial cells. Apoptosis, 2014; 19(5):776-788.
90. Li G, Tang J, Du Y, Lee CA, Kern TS; Beneficial effects of a novel RAGE inhibitor on early diabetic retinopathy and tactile allodynia. Molecular vision, 2011;17: 3156–3165.
91. Kaji Y, Usui T, Ishida S, Yamashiro K, Moore TC, Moore J, *et al.*; Inhibition of diabetic leukostasis and blood-retinal barrier breakdown with a soluble form of a receptor for advanced glycation end products. Investigative ophthalmology & visual science, 2007; 48(2):858-865.
92. Yamagishi SI, Matsui T, Nakamura K, Inoue H, Takeuchi M, Ueda S, *et al.*; Olmesartan blocks advanced glycation end products (AGEs)-induced angiogenesis in vitro by suppressing receptor for AGEs (RAGE) expression. Microvascular research, 2008; 75(1):130-134.
93. Maeda S, Matsui T, Ojima A, Takeuchi M, Yamagishi SI; Sulforaphane inhibits advanced glycation end product–induced pericyte damage by reducing expression of receptor for advanced glycation end products. Nutrition Research, 2014; 34(9):807-813.

94. Yamagishi SI, Matsui T; Advanced glycation end products (AGEs), oxidative stress and diabetic retinopathy. CurrentPharmaceutical Biotechnology, 2011; 12(3):362-368.
95. Peponis V, Papathanasiou M, Kapranou A, Magkou C,Tyligada A, Melidonis A, *et al.*; Protective role of oral antioxidant supplementation in ocular surface of diabetic patients. British journal of ophthalmology, 2002; 86(12):1369-1373.
96. Pazdro R, Burgess JR; The role of vitamin E and oxidative stress in diabetes complications. Mechanisms of ageing and development, 2010; 131(4):276-286.
97. Lin J, Bierhaus A, Bugert P, Dietrich N, Feng Y, Vom Hagen F, *et al.* ; Effect of R-(+)-α-lipoic acid on experimental diabetic retinopathy. Diabetologia, 2006; 49(5):1089-1096.
98. Lukinova N, Iacovelli J, Dentchev T, Wolkow N, Hunter A, Amado D, *et al.* ; Iron chelation protects the retinal pigment epithelial cell line ARPE-19 against cell death triggered by diverse stimuli. Investigative ophthalmology & visual science, 2009; 50(3):1440–1447.