Molecular pathobiology of Diabetic Retinopathy

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Abstract

Background: Diabetes and its associated complications constitute a huge global health menace. Hyperglycemia induces alterations in several signaling pathways, which ultimately culminate in the development of various complications that includes Diabetic Retinopathy (DR). DR, a disease of retina is a major cause of blindness among diabetics.
Challenges: Although the precise mechanism involved in the pathogenesis of DR is broadly unclear due to its multifactorial nature, the contribution of oxidative stress and inflammation occupy the central stage.
Conclusion: In this review, the prevalence, clinical and pathobiological characteristics of DR and the possible cross-talkbetween the different contributing pathways involved in its development are described.

Introduction

Diabetes mellitus or simply diabetes is a lifelong progressive disease that results from body's inability to produce insulin, use of insulin to its full potential or both, and is characterized by high circulating glucose (Hyperglycemia)[3]. This disease has reached epidemic proportion and has become one of the most challenging health problems of the 21st century. Indeed, by the year 2010, the total number of people with diabetes is projected to reach 221 million worldwide[7] and by the year 2025, this number is estimated to reach 334 million. There are two distinct forms of diabetes, type 1, juvenile or Insulin-Dependent Diabetes Mellitus (IDDM) and type 2, adult onset or Non Insulin-Dependent Diabetes Mellitus (NIDDM)[3]. Type 2 diabetes accounts for 90 percent of all diabetes cases. Diabetes is a chronic heterogeneous metabolic disorder with complex pathogenesis. The sustained hyperglycemia in diabetes attacks both microvessels and macrovessels throughout the body that lead to complications, such as cardiovascular disease, renal failure, retinopathy and peripheral and autonomic neuropathy[16][34].

As a consequence of these complications, diabetes is the leading cause of blindness and visual impairment, noninjury amputation, and end-stage kidney disease in adults at developed countries. The patients with diabetes develop cataracts in an earlier age, and are nearly twice as likely to get glaucoma compared to nondiabetics[100]. These complications occur in both type 1 and type 2 diabetes irrespective of the fact that type 1 and type 2 diabetes originate from different pathogenetic causes thereby indicating that there is a significant association between hyperglycemia and the diabetic microvascular complications in both diabetes types[12].

Although, all the features of diabetic retinopathy may be found in both types of diabetes, but characteristically, the incidence of the main causes of vision loss, macular edema and retinal neovascularization is quite different for each type of diabetes[5].

Diabetic Retinopathy

Diabetic retinopathy (DR), a disease of the retina, is the most common and specific microvascular complication of diabetes[83] . It is the major cause of blindness among people in working age and is a leading cause of visual loss in older patients in developed countries[106], thus representing a major socioeconomic problem. Diabetic retinopathy is present to some degree in nearly all individuals who have had diabetes for more than 15 years, regardless of the type of diabetes. It is a duration-dependent disease that develops in stages; the incidence of retinopathy is rarely detected in the first few years of diabetes, but the incidence increases to 80% by 10 years, and to virtually 95% by 25 years of type 1 diabetes[6]. However, the overall incidence of DR is slightly less common in type 2 diabetes and nearly 75 per cent of type 2 diabetes patients develop this disease after 15 yr duration of diabetes[57], but it still remains the most frequent microvascular complication suffered by these patients. The pathogenic basis of diabetic retinopathy is not wholly understood at the cellular and molecular level but it is clear that the pathogenesis of diabetic retinopathy is highly complex and multifactorial. It is now known through major international epidemiological trials that hyperglycemia is a principle and underlying cause of DR in both IDDM and NIDDM[12]. Hyperglycemia is responsible for not only microvascular[12] disease but also of macrovascular complications[71] in both forms of diabetes. Hyperglycemia causes retinal damage via many acute (and repeated) and also cumulative long-term changes (Fig. 1).
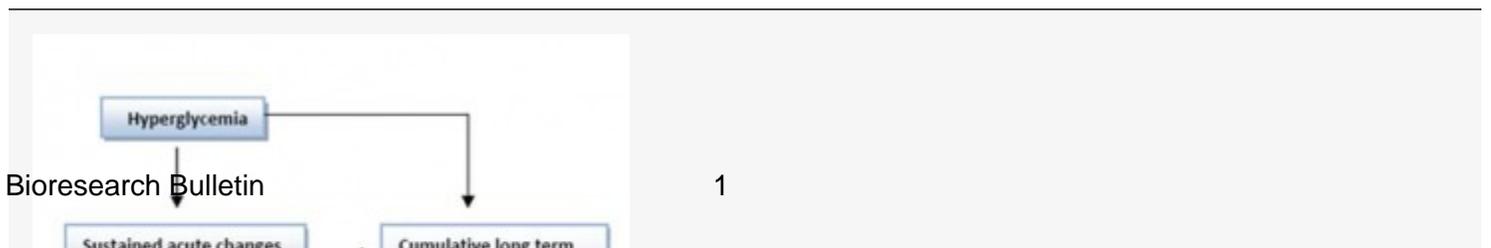
Fig. 1: Hyperglycemia induced retinal damage via sustained acute and cumulative changes in cellular metabolism and subsequent cumulative changes in stable macromolecules that ultimately lead to tissue damage.

Diabetic retinopathy and vascular damage

In diabetic retinopathy, most cells of the retina are affected by the metabolic abnormalities of diabetes[76], but the sight-threatening manifestations of diabetic retinopathy are ultimately attributable to capillary damage. The blood vessels of retina have tight junctions that protect them from leaking, but sustained high glucose damages the tight junctions and the vessels become leaky that cause the blood vessels to swell and leak fluid. The damaged microvasculature allows the fluid or blood to seep into the retina resulting in the breakdown of blood–retinal barrier that causes the swelling of the retina or macular edema[24,44]. The capillaries of retina are lined with endothelial cells that are responsible for maintaining the blood retinal barrier, and are supported with an equal number of pericytes (microvascular mural cells) that help provide tone to the vessels.

However, in diabetes, the ratio of endothelial cells to pericytes is altered to 4:1[95]. As the disease progresses, the endothelial cells try to repair the damaged vessel by multiplying on the inner side of the vessel wall blocking the capillaries. Due to progressive dysfunction, the capillaries die prematurely leading to ischemia that is followed by new vessel growth or neovascularization. New capillaries start to grow from the surface of the retinal veins towards the center of the eye with no support, and ultimately lead to the detachment of the retina[37,63,610]. The leukocytes become less deformable, and retinal leukostasis is increased affecting endothelial function[52]. The macular edema caused due to a breakdown in the blood–retinal barrier, is one of the earliest clinically detectable signs of DR and of all the clinical features, macular edema is most closely correlated with the degree of vision loss[84].

Classification of Diabetic Retinopathy

The diabetic retinopathy is broadly classified as either Non Proliferative Diabetic Retinopathy (NPDR) or Proliferative Diabetic Retinopathy (PDR). The classification of diabetic retinopathy has evolved as our understanding of diabetic eye disease has increased. Nonproliferative diabetic retinopathy, also known as background retinopathy, occurs when there are only intraretinal microvascular changes, such as altered retinal vascular permeability and eventual retinal vessel closure[80]. It is further divided into NPDR with maculopathy, NPDR without maculopathy and pre-proliferative retinopathy. NPDR indicates progressive ischemia in the retina and an increased risk for the development of PDR and blindness.

The prominent clinical features of NPDR are often classified as red lesions, such as microaneurysms, dot or blot hemorrhages, and intraretinal microvascular abnormalities, or bright lesions, such as lipid or lipoprotein hard yellow exudates, and superficial retinal infarcts (cotton-wool spots) [75,90]. Microaneurysms are saccular outpunching of the capillary wall in the region of vascular occlusions. These are visible as small round dark red dots on the retinal surface. Hemorrhages and exudates result from the damaged vasculature. Increase in retinal blood flow and vasodilation occurs early in the course of the disease, followed by the microvascular leakage and occlusion, and histologic data corroborates the clinical evidence[32,70]. Maculopathy is defined as the presence of edema and/or haemorrhages and/or exudates and/or retinal thickening within 500 m (i.e., 1 disc diopter) of the fovea, with or without visual loss. Preproliferative diabetic retinopathy is the stage before the onset of neovascularization and is characterized by:

(a) Extensive retinal haemorrhages,

(b) Marked venous beading,

(c) Numerous cotton wool spots or retinal infarcts,

(d) Intra-Retinal Microvasculature Abnormalities [[IRMAs, a term chosen so as to be neutral about whether these abnormal vessels represent intraretinal new vessels or dilated preexisting vessels], and

(e) Marked retinal ischemia as evidenced by capillary drop outs in the fundus fluorescein angiogram. Neovascularization is not a component of the nonproliferative phase.

However, in advanced NPDR, nonperfusion of the retina may develop and lead to the proliferative phase[80].

Proliferative diabetic retinopathy continues to be a major cause of blindness throughout the world. It is characterized by new vessels (neovascularization), preretinal haemorrhage, vitreous haemorrhage, vitreoretinal traction, localized retinal detachment and sometimes fibrous bands proliferating on the retinal surface and these are the primary complications leading to vision loss[26]. In both nonproliferative and proliferative diabetic retinopathy, macular edema can occur as increased retinal vascular permeability leads to accumulation of fluid in the retinal area serving central vision[80]. There is accumulated evidence indicating that only the Non Proliferative stage of Diabetic Retinopathy (NPDR) is directly due to the systemic disease and associated hyperglycemia and other metabolic alterations. Proliferative retinopathy occurs in diabetic eyes only after the development of widespread ischemia due to capillary closure. Neovessels in the retina are a direct result of retinal ischemia and not influenced by the diabetic metabolic control. Its course and management are not different from other situations in the retina where there is abnormal new vessel formation[54].

Oxidative stress in Diabetic Retinopathy

The pathogenic basis of diabetic retinopathy is not wholly understood but various studies have shown the important, in fact, the central role that oxidative stress and inflammation play in the pathogenesis of this disease. Oxidative stress refers to the condition in which there is a serious imbalance between the production of oxidants (reactive oxygen species (ROS) and reactive nitrogen species (RNS)), and antioxidant defense, leading to potential tissue damage[97]. Diabetes results in increased oxidative stress and increased oxidative stress is one of the key regulators in the development of diabetic complications. The possible sources of oxidative stress in diabetes might include auto-oxidation of glucose, disruption of the mitochondrial electron transport chain, shifts in redox balances, decreased tissue concentrations of low molecular weight biologically existing antioxidants such as reduced glutathione (GSH), vitamin E, vitamin C and ?-carotene and impaired activities of antioxidant defense enzymes such as superoxide dismutase (SOD), glutathione reductase, glutathione peroxidase and catalase, that are responsible for scavenging free radicals and maintaining redox homeostasis and as such superoxide levels are elevated in the mitochondria[113][13456061636566858855]. The retina has a high content of polyunsaturated fatty acids and has the highest oxygen uptake and glucose oxidation relative to any other tissue. This phenomenon renders retina more susceptible to oxidative stress[8].

Hyperglycemia induced ROS generation is considered as a causal link between elevated glucose and the other metabolic abnormalities important in the development of diabetic complications and normalizing mitochondrial ROS is shown to prevent glucose induced metabolic abnormalities that are postulated in the pathogenesis of diabetic complications including the diabetic retinopathy[861920]. The correlation between hyperglycemia, changes in the redox homeostasis, and oxidative stress have been suggested to be the key events in the pathogenesis of diabetic retinopathy. Animal studies have demonstrated that oxidative stress contributes not only to the development of diabetic retinopathy but also to the resistance of retinopathy to reverse after good glycemic control is reinstituted—the metabolic memory phenomenon[62].

Resistance of diabetic retinopathy to reverse is probably attributed to accumulation of damaged molecules and ROS that are not easily removed even after good glycemic control is reestablished. Since oxidative stress represents an imbalance between excess formation and/or impaired removal of ROS, the antioxidant defense system of the cell is a crucial part of the overall oxidative stress experienced by a cell. Further, the cell is equipped with intracellular antioxidant, GSH; GSH is probably the most important defense the cell is equipped with. It can act as an ROS scavenger and modulate intracellular redox state[78]. The levels of this intracellular antioxidant are decreased in the retina in diabetes[56], and the enzymes responsible for its metabolism are compromised[5964]. Apart from the antioxidant defense enzymes, nonenzymic antioxidants such as vitamin C, vitamin E, and ?-carotene that exist biologically for the regulation of redox homeostasis are also depressed during hyperglycemia induced oxidative stress[35]. The consequences of chronic oxidative stress are many that include damage to biological macromolecules such as DNA, lipids, proteins, and carbohydrates, disruption in cellular homeostasis, and generation of other ROS. These processes create further damage that result in many pathological processes of clinical interest that includes the diabetic retinopathy[25]. However, the exact mechanism by which oxidative stress could contribute to the development of diabetic complications still remains to be clarified.

Role of other signalling pathways

Oxidative stress, besides creating a vicious cycle of damage to macromolecules by amplifying the production of more ROS, also activates other metabolic pathways that are detrimental and promote the development of diabetic retinopathy. These include the Advanced Glycation End products (AGE) pathway[15], the polyol pathway[56], the hexosamine biosynthesis pathway[31], the de novo synthesis of diacylglycerol (DAG) leading to the overactivation of several isoforms of protein kinase C [Protein Kinase C (PKC) pathway][7310268110] besides several other that include alteration in the expressions of Vascular Endothelial Growth Factor (VEGF)[21] and insulin-like growth factor-1 (IGF-1)[27], and elevation in mitochondrial overproduction of superoxide and mitochondrial dysfunctions (Fig. 2)[60].

Advanced glycation end products (age) pathway

Advanced Glycation End products (AGE) are a heterogeneous group of compounds that are produced as a result of non-enzymatic condensation reactions between reducing sugars and ?-amino groups or N-terminal groups of amino acids, preferentially lysine and arginine[109]. The heterogeneity of AGEs comes from the fact that majority of these products come from an array of precursor molecules with variable chemical structure. The intermediates that are mostly involved in the formation of AGEs include dicarbonyls such as 1-, 3-, or 4-deoxyglucosones, glyoxal, and methylglyoxal. These are highly reactive intermediates that are upregulated during hyperglycemia, and react with proteins and form intramolecular or intermolecular AGEs[104][105]. The AGEs are also formed on the amino groups of lipids and DNA. AGE formation can lead to the alterations in function, activity, and degradation of both intracellular and extracellular proteins via chemical rearrangement and intramolecular and intermolecular cross-linking. For example, it causes the cross-linking of proteins such as collagen or elastin in the wall of blood vessels thereby altering its structural integrity.

Besides being able to crosslink a number of proteins and alter their physical properties, AGEs interact with a specific receptor, RAGE on vascular cells to activate multiple signaling pathways (e.g. protein kinase C [PKC] and MAP kinase pathways) and activate nuclear factors (e.g. NF-kB and Cyclic AMP-Responsive Element-Binding protein; CREB, resulting in an increase in ROS production and elaboration of inflammatory factors that cause further damage to the cells[115] . There is an increased accumulation of AGE and its receptor, RAGE in the retinal microvasculature in diabetes[103]. AGEs are irreversibly formed and accumulate within retinal capillary cells in the late stages of retinopathy. The AGEs also increase nitrative stress in the retinal vascular cells and initiates a sequence of events leading to retinal capillary cell apoptosis via activation of NF-kB and caspase-3 [61]. t may also lead to nitration of proteins which can inactivate mitochondrial and cytosolic proteins; disrupt protein assembly and functions, and increase apoptosis, ultimately leading to pathological consequences and cellular damage[23].

Polyol Pathway

The polyol pathway becomes active under hyperglycemic conditions[41][87]. Elevation in glucose activates the enzyme Aldose Reductase (AR), the first and rate-limiting enzyme in the pathway which reduces glucose to sorbitol using NADPH as a cofactor. As NADPH is used, increased aldose reductase activity (polyol pathway) can therefore alter NADPH/NADP balance and decrease the availability of NADPH cofactor for glutathione reductase, which is critical for the maintenance of the intracellular pool of antioxidant reduced glutathione (GSH). This decreases the capability of cells to respond to oxidative stress[12]. Sorbitol produced from glucose by AR is then metabolized to fructose by sorbitol dehydrogenase that uses NAD^+ as a cofactor. Since the conversion of sorbitol to fructose requires reduction of NAD^+ to NADH, sorbitol dehydrogenase leads to an increased ratio of $NADH/NAD^+$, a condition termed "pseudohypoxia" that has been linked to a multitude of metabolic and signaling changes known to alter cell function[112]. The increased amounts of NADH lead to higher levels of oxidized triose phosphates, precursors of AGEs and of diacylglycerol (DAG) through ?-glycerol-3-phosphate. Thus, activities through the AGE and PKC pathways are enhanced[26]. The excess NADH may become a substrate for NADH oxidase, and this would lead to the generation of intracellular oxidant species[72]. Sorbitol is a polyhydroxylated, and strongly hydrophilic alcohol that does not diffuse readily through cell membranes and therefore accumulates intracellularly with possible osmotic consequences[30].

Hexosamine Pathway

Another pathway that plays an important role in the development of hyperglycemia induced diabetic complications including the diabetic retinopathy is the hexosamine pathway[99][58]. This pathway is yet another outcome of hyperglycemia and increased superoxide levels that are characteristic of diabetes[14]. The inhibition of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and hyperglycemia-induced mitochondrial superoxide overproduction plays an important role in the activation of the hexosamine pathway, presumably by diverting the upstream metabolite fructose-6-phosphate from glycolysis to glucosamine formation[31]. Inhibition of GAPDH can result in increased levels of glycolytic metabolite, glyceraldehyde-3-phosphate that can activate the AGE pathway by activating intracellular AGE precursor methylglyoxal[78][110]. This pathway results in the production of dUDP-N-acetyl glucosamine which is a substrate used for the post-translational modification of intracellular factors including transcription factors[31]. In addition, GAPDH can also be modified by direct glycation and by nitration[50][14] and over-expression of manganese SOD (MnSOD) decreases the activation of GAPDH.

Protein kinase C (PKC) pathway

The Protein Kinase C (PKC) pathway also plays an important role in the pathogenesis of diabetic retinopathy[49][109][60]. Hyperglycemia can activate the PKC pathway directly via diacylglycerol (DAG) synthesis and indirectly through production of ROS or activation of the AGE/RAGE pathway and polyol pathway products[91][102][101][62][94]. PKC has many isoforms and all the classic and novel PKC isoforms are activated by DAG, but primarily the ? and ? isoforms appear to be involved in diabetes[67].

Activated PKC, particularly PKC ? can bring about a variety of changes characteristic of diabetic retinopathy that include increasing vessel permeability, blood flow by virtue of its ability to alter the balance of vasodilatory/ vascoconstrictive factors [e.g. endothelial nitric oxide synthase(eNOS), endothelin-1(ET-1)], alteration of hormone and growth factor receptor recycling, stimulation of neovascularization, induction of adhesion molecule expression [e.g. vascular cell adhesionmolecule-1(VCAM-1)] endothelial proliferation and apoptosis, and regulating the action of several factors such as VEGF, IGF-1, Connective Tissue Growth Factor (CTGF) and transforming growth factor ?1(TGF ?1)[89,114,110,10,194]. De novo synthesis of DAG comes largely from glycolytic intermediates and stepwise acylation of glycerol-3-phosphate. DAG can also be synthesized through the phospholipase pathways activated by growth factors, cytokines, and hormones, such as angiotensin II, which are elevated in diabetes[77,111,119].

Several of these pathways may also mediate oxidative stress creating an interrelated connection with oxidative stress as well as with other pathways that amplify tissue damage even further. However, it is difficult to pinpoint which pathway/s is/are critical to the development of diabetic retinopathy. More likely, no single metabolic dysfunction seems to be the sole contributor and possibly all pathways interact to create the histopathology changes seen in diabetic retinopathy.

Inflammation in Diabetic Retinopathy

The inflammatory nature of Diabetic Retinopathy (DR) was first suggested by the 1960s finding that there is a lower incidence of DR among diabetic patients taking salicylates for rheumatoid arthritis[93]. This has been complemented by the subsequent work that has provided further support for this linkage and has identified many characteristic inflammatory features that accompany the progress of DR in patients and in animal models.

The Inflammatory features that characterize diabetic retinopathy include increased blood flow and vascular permeability, tissue (macular) edema, neovascularization[9], increased expression of inflammatory mediators[45,73,94,11,61,82,27,99,21,07,108,29,46], accelerated retinal neural[11] and microvascular cell death, macrophage infiltration[33,22], microglial cell activation[98,117,69], increased leukocyte adhesion[81,52], complement activation[118], Fas ligand upregulation[53] and acute-phase response protein expression[43].

The inflammatory response in early diabetes includes cytokine release, which activates key transcriptional regulators, including nuclear factor kappa B (NF-?B). NF-?B is a widely expressed inducible transcription factor that is an important regulator of many genes involved in mammalian inflammatory and immune responses, proliferation and apoptosis. It regulates expression of many proinflammatory molecules at the level of gene transcription including inducible Nitric Oxide Synthase (iNOS), cyclooxygenase 2 (COX-2), interleukin-1 (IL-1), Tumor Necrosis Factor alpha (TNF-?) and intracellular adhesion molecule (ICAM). The important role of NF-?B in the pathogenesis of early stages of diabetic retinopathy is well supported by the fact that the inhibition of proteins whose expression is regulated by NF-?B (such as iNOS and ICAM as already mentioned) inhibit diabetes induced degeneration of retinal capillaries. Also, it is now well established that the compounds known to inhibit NF-?B likewise inhibit the development of the retinopathy. For example, several different antioxidants which inhibit the development of capillary degeneration and pericyte loss in retinas of diabetic rats[63] also inhibit the diabetes-induced activation of retinal NF-?B[96]. Likewise, low-intermediate doses of salicylates (aspirin, sodium salicylate, and sulfasalazine) which inhibited NF-?B activation in retinas of diabetic rats, also inhibited expression of inflammatory mediators like iNOS and ICAM-1, and capillary degeneration and pericyte loss in those animals[63,66]. Aspirin is known to inhibit the production of prostaglandins also, but salicylate and sulfasalazine have much less of this activity, suggesting that the common action of these three salicylates to inhibit retinopathy in diabetes was primarily not mediated by inhibition of prostaglandins. The proinflammatory molecules such as inducible nitric oxide synthase (iNOS), cyclooxygenase 2 (COX-2), interleukin-1 (IL-1), tumor necrosis factor alpha (TNF-?) and Intracellular Adhesion Molecule (ICAM) whose expression is regulated by NF-?B contribute in their own capacity towards the development of diabetic retinopathy. The increased expression of cell adhesion molecules, such as ICAM-1, for example, promotes the attraction and adhesion of leukocytes to the retinal vascular wall, a process known as leukostasis.

Leukostasis is linked to increased vascular permeability possibly through the mechanisms that may involve direct action on tight junction disruption [28,17] as well as local upregulation of Vascular Endothelial Growth Factor (VEGF), either from the hypoxia induced by nonperfusion or from release by the leukocytes themselves[48,38,42,47]. However, it is the concomitant increase in endothelial cell injury and subsequent cell death that is mainly responsible for the process[51,52,53].

Advances & hurdles in the treatment of DR

Recent advances in elucidating the molecular mechanisms of disease have led to the development of an unprecedented number of potentially disease-modifying pharmacologic agents for DR. To a greater or lesser extent, all of the proposed treatments for DR inhibit processes that increase fluid leakage, cellular proliferation, or inflammation independently of hyperglycemic control. Treatments currently proposed for DR and their associated effects are grouped into 7 drug classes: antioxidants, Aldose Reductase Inhibitors (ARIs), growth hormone inhibitors, renin-angiotensinaldosterone system inhibitors, steroids, Vascular Endothelial Growth Factor (VEGF) inhibitors, and Protein Kinase C (PKC) inhibitors.Ideally, it is desirable for a drug to have a highly specific effect on abnormal physiological processes while leaving normal physiological processes un-impaired. However, numerous cytokines, growth factors, and other substances contribute to each aspect of retinal tissue damage in DR and they come from different metabolic pathways. Furthermore, some of these pathways and factors are essential to normal physiologic functioning. Thus, suppressing abnormal molecular activity but still permitting normal physiologic

function is a significant challenge in designing disease-modifying drugs for DR.

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