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Review on Early Neurodegenerative Changes in Diabetic Retinopathy

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Abstract Diabetic retinopathy (DR) is a common complication of diabetes which is the 5th primary cause of blindness globally and leading cause of blindness in the developed countries in adults at working age. This is a metabolic disorder that produces various dysfunctions in the body through Oxidative stress induced by chronic hyperglycemia. It has a direct outcome on metabolism within the neural retina, foremost to an increase in apoptosis, which in turn causes breakdown of the blood-retinal barrier. The clinical hallmark of DR includes increased vascular permeability, leading to edema and endothelial cell proliferation. Even though, there is emerging evidence to suggest that retinal neurodegeneration is an early event in the pathogenesis of DR, which participates in the development of microvascular abnormalities. Therefore, this review underlying mechanisms leading to neurodegeneration and the identification of the mediators between neurodegeneration and Diabetic retinopathy will be essential for the development of new therapeutic strategies.

Keywords: *Diabetic retinopathy, Neurodegeneration, oxidative stress, blood-retinal barrier*

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1. Introduction

Diabetes is an epidemic and its prevalence is increasing in all aspects of society. While some populations are at higher risk for diabetes and may be more prone to its complications. This is a disease that has no boundaries and can affect anyone at any point in one's lifetime [1]. The total number of people with diabetes is projected to rise from 171 million in 2000 to 366 million in 2030 [2].

Diabetes is a major cause of mortality and morbidity [3,4]; Patients with long-standing diabetes may develop complications affecting the eyes, kidneys or nerves (microvascular complications) or major arteries. The major arteries are affected in people with diabetes, causing a substantial increase in both in coronary artery disease and strokes as well as peripheral vascular disease [5].

The above idea is also supported by cockram [6] by stating that most the important chronic complications of diabetes were those affecting blood vessels and nerves such as microvascular complications including retinopathy, neuropathy and nephropathy or macrovascular complications comprising coronary heart, cerebrovascular and peripheral vascular diseases.

Diabetes is also considered to be a risk factor for neurodegenerative diseases such as Alzheimer's disease and other [7]. It has been proposed that the risk of stroke and other types of cerebrovascular accidents may increase in diabetes and structural and/or functional changes observed in the microvasculature in diabetic neuropathy may be involved in the resulting nerve damage [8]. In addition it has been reported that diabetes increases the cerebrospinal fluid pressure [9], which is responsible for the excessive retention or production of CSF from epithelial cells of choroid plexus [10]. This is a metabolic disorder that produces various dysfunctions in the body through Oxidative stress induced by chronic hyperglycemia that play a key role in both microvascular and macrovascular complications of diabetic patients [11].

2. Diabetic Retinopathy

Diabetic retinopathy (DR) is a disease resulting from the complications of diabetic mellitus(DM). It is complications in the retina, where the photoreceptor cells, the neuronal elements responsible for vision, are located. [12].

DR is the 5th leading cause of blindness globally, while it is the leading cause of blindness in the developed countries in adults at working age. The global prevalence of DR is projected to exceed 200 million individuals in 2012 [12]. The prevalence rate for retinopathy for adults aged 40 years and older in the United States is 3.4%. Duration of diabetes is a major risk factor associated with the development of diabetic retinopathy. After 5 years, approximately 25% of type 1 patients have retinopathy, and after 15 years, 80% have retinopathy [13].

The duration of uncontrolled glycemia in DM is revealed by the increase of glycate hemoglobin (HbA1c) and it is risk factor in the development of DR [14]. Several inter-related pathways, such as oxidative stress, polyol pathway, and protein kinase C (PKC) activation, have been shown to contribute to diabetes-induced retinal

damages [15]. In addition, diabetic retinopathy is recently recognized as a chronic low-grade inflammatory disease [16].

Today DR has been defined based on lesions that are clinically demonstrable, and all of those have been vascular in nature [17], which becomes clinically obvious after 10-15 years of diabetes in humans [18]. One of the earliest clinical signs detected in DR is increased vascular permeability due to the collapse of the blood-retinal barrier, which causes macular edema [19]. It was followed later, microaneurysms, exudates, and, finally, vascular proliferation [20]. These clinical findings, the macular edema are mostly correlated with the degree of visual loss [21].

In diabetes, chronic exposure to high blood glucose, hyperglycemia, leads to the formation of glucotoxins and other mediators leading to endothelial hyperpermeability, activation of inflammatory cells, and abnormal expression of vascular factors [22]. Vasicular Endothelial growth factor (VEGF) is becoming more evident in diabetic retina, in addition to the vascular changes there are other degenerative changes of the neural part of the retina[23].

3. Neurodegeneration in Diabetic Retina

In neural changes either of the following are seen neuronal loss, gliosis, and the presence of aggregates of misfolded or aberrant proteins [24,25]. The neurons in degenerative diseases die generally by apoptosis. A failure of the neurons to cope with oxidative stress likely promotes neurodegenerative changes. Oxidative stress renders the neurons vulnerable to the slowly evolving effects of excitatory neurotransmitters. Persistent over excitation of the neurons ultimately leads to their deaths [26].Neurodegenerative begins long before patients' experience any symptoms, which are noticed only when many cells are irreversibly damaged and cease to function, so that the actual onset of disease precedes clinical manifestations by many years [24].

Degenerative changes occur beyond the vascular cells of the retina. These changes include: increased apoptosis, glial cell reactivity, microglial activation and altered metabolism of glutamate. When they occur together, they can be considered as neurodegenerative and could explain some of the functional deficits in vision that occur soon after the onset of diabetes [23]. The gradual loss of neurons in the diabetic retina suggests that progression of the disease is ultimately irreversible, since these cells cannot be replaced [18].

Diabetes is increasingly recognized that ongoing early changes in the retina neuronal and glial cells may initiate pathological changes prior to the development of clinically detectable microvascular damage [18,27] but the blood vessels in the retina and the choroid do not show any abnormality in the early stage of the disease [28].

4. Diabetic Retinopathy and Oxidative Stress

Generation of highly Reactive Oxygen Species (ROS) is an integral feature of normal cellular function like mitochondrial respiratory chain, phagocytosis, arachidonic acid metabolism, etc. Their production however, multiplies several folds during pathological conditions [29].

Damage due to free radicals caused by ROS leads to several damaging & effects as they can attack lipids, protein/ enzymes, carbohydrates, and DNA in cells and tissues. They induce undesirable oxidation, causing membrane damage, protein modification, DNA damage, and cell death induced by DNA fragmentation and lipid peroxidation [30].

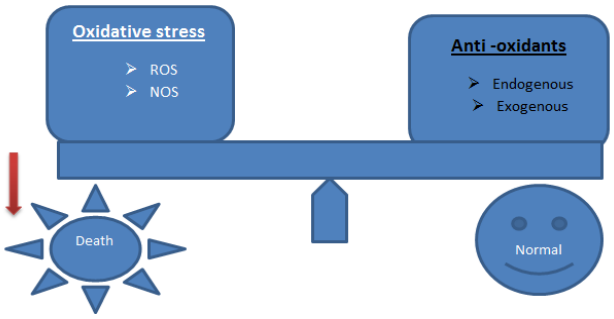

Figure 1. Balance of ROS generation and ant-oxidative systems

Experimental diabetes induced significant increases in oxidative stress, retinal neuronal cell death, and vascular permeability [31,32]. These effects were associated with increased levels of tumor necrosis factor (TNF)- α, vascular endothelial growth factor (VEGF), and intercellular adhesion molecule (ICAM)-1 and activation of p38 MAP kinase, [33]. Similar metabolic changes were seen in experimental rats & mice, increased oxidative stress, PKC activity, and nitric oxide (NO) level in retina [34].

Activation of glutamate receptors in turn leads to activation of nitric oxide synthase and excess accumulation of superoxides and NO, causing lipid peroxidation, mitochondrial dysfunction, DNA damage, and the eventual cell death [35,36].

Oxidative stress, described as a disturbance in the pro-oxidant–antioxidant balance in favor of the pro-oxidant, can be involved in the pathophysiology of several human diseases [37]. An imbalance of both systems due to either excessive production of ROS or reduced antioxidant defense leads to oxidative stress. See fig below [38].

There are many possible pathways of oxidative stress that can be associated with hyperglycemia and DR.

Autoxidative glycosylation' describes as a role of reducing sugars as catalysts of oxidative chemical modification and cross-linking of proteins and it increases in the presence of hyperglycemia. During this reaction the following are formed superoxide, the hydrogen radical and hydrogen peroxide.. Some of these products undergo further chemical rearrangements to form irreversible, advanced glycosylation end products (AGEs). These can cause oxidative damage to neighbouring. Therefore, autoxidative glycosylation is a possible mechanism for the production of free radicals, leading to fragmentation of proteins and oxidation of associated lipids [39,40].

The interaction of AGE with its receptor RAGE alters intracellular signalling, gene expression, release of proinflammatory molecules, and production of reactive oxygen species that may contribute towards the pathology of DR [41].

Advanced glycosylation index(AGI), a simple measure of AGEs, was shown to be increased in diabetic patients compared to normal subjects, and this increase strongly correlated to the development and progression of DR. AGI

was significantly increased in diabetic subjects with different severities of retinopathy. More importantly, AGI levels were significantly associated with both the non-proliferative stage and the proliferative stage of DR [42]. The main risk factors for DR in this population were uncontrolled glycemia and duration of diabetes [14]. Increased formation of advanced glycation end products (AGEs) is related to hyperglycemia and their pathological relationship could explain many of the changes observed in diabetes-related complications [43].

In hyperglycemic, AGEs lead to early inner retinal neuronal dysfunction. Here, AGEs were also localized to the vitreous cavity and internal limiting membrane (ILM) of the retina, where they were intimately associated with the footplates of RAGE-expressing Muller cells. Furthermore, AGE accumulation was increased within the retinal extracellular matrix and attenuation of the RAGE axis with soluble RAGE ameliorated neuronal dysfunction and reduced the development of capillary lesions in these mice [44].

Lipid peroxidation- reduced anti-oxidant defense; it has been suggested that the reduction in the anti-oxidant state contributes to the development of oxidative stress in diabetes. A reduced total anti-oxidant activity was demonstrated in both type 1 and type 2 diabetes [45]. It is generally assumed that, in the presence of increased oxidative stress, these circulating anti-oxidants are consumed and that consequently plasma concentrations fall [46].

Under diabetic conditions, reactive oxygen species (ROS) are produced via glucose autoxidation and also via non-enzymatic protein glycation in various tissues [47]. ROS are associated with different complications (e.g., eye, kidney, and nerve damage [47,48]. Although the induction of the glycation reaction in diabetes was originally found in neural cells and lens crystalline, which are also known targets of diabetic complications [47].

Diabetes mitochondrial dysfunction induces ERstress, Communication between the endoplasmic reticulum (ER) and mitochondrion is important for bioenergetics and cellular survival. The ER supplies Ca^{2+} directly to mitochondria via inositol 1,4,5-trisphosphate receptors (IP3Rs) [49]. Mitochondrial Ca^{2+} contributes to many physiological events, including bioenergetics, neuroplasticity, and cell death. During oxidative stress Mitochondrial Ca^{2+} overloading leads to apoptosis when cells are under pathological conditions [50]. The ER is a multifunctional organelle that controls protein translation as well as Ca^{2+} homeostasis [51]. Over expression of the enzyme responsible for scavenging mitochondrial superoxide prevents these diabetes-induced mitochondrial alterations and histopathology characteristic of diabetic retinopathy [52]. Retinal mitochondria become dysfunctional and start to leak cytochrome c into the cytosol, and superoxide levels are elevated [53]. Diagram below shows causes of DR is secondary to different cascades that form oxidative stress in the diabetic retina.

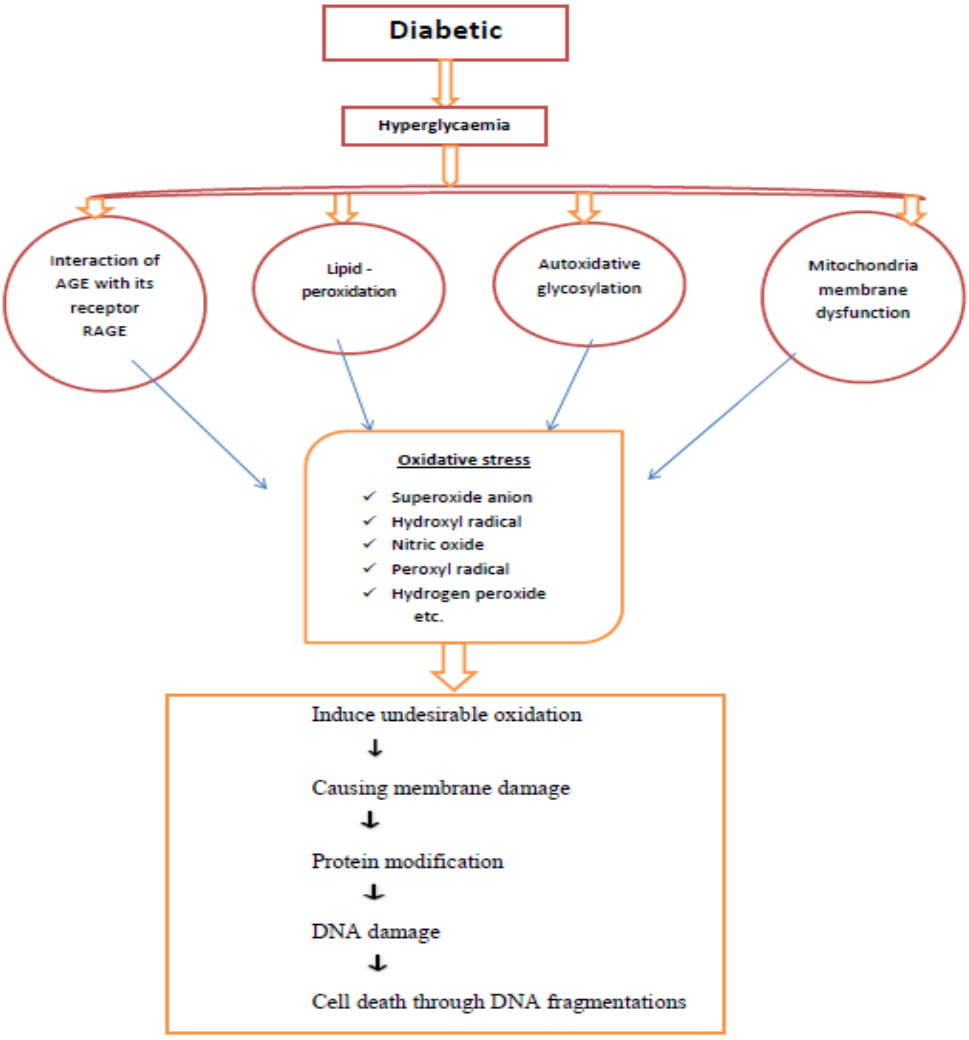

Figure 2. Proposed oxidant generating pathways and cell death in diabetes

In diabetic rats' retina antioxidant capacity and the levels of intracellular antioxidant, GSH (reduced form of glutathione) levels were decreased and oxidatively modified DNA (8-OHdG) and nitrotyrosine were increased in the retina of diabetic rats. The levels of interleukin-1β (IL-1β) and VEGF were elevated, and the nuclear transcription factor (NF-kB) was activated [53]).

5. Neural changes in DR

Different experimental researches show that there are morphological changes in the neural part of diabetic retinopathy; these changes are in thickness, number of neural cells, GFAP reactivity and other; after the induction of diabetes In the 4 weeks the thickness of the of innerplexiform (IPL) and nuclear layers which was significantly decreased [23,55,56]. In the 8th weeks after onset of diabetes both IPL and inner neural layer (INL) significantly become thinner in diabetic retina compared with control rats. Park [57]also shown report that thinning of the inner nuclear layer and also marked thinning of the outer nuclear layer by 24 weeks after the onset of diabetes.

Retinal ganglion cells (RGC) count decreased significantly in both the 4 and 8 weeks after induction of diabetes [23,56]. Morphometric analysis of retinal cross sections of mice that had been diabetic 14 weeks showed significant fewer cells(20%-25%) in the ganglion cell layer compared with age matched control mice[58]. Neurons in the GCL and the inner part of the INL were moderately reduced after one month of diabetes, corresponding with up-regulation of glial fibrilary acidic protein (GFAP) in Müller cells [59].

The RGC loss and decrease in the thickness of IPL and INL in diabetic rats and mice in the early stages of diabetes may be caused due to retinal neural cells death; As both apoptosis and necrosis occur as a degenerative changes of neural retina in ongoing diabetic retinopathy [57].

Glial cells reactivity is seen in diabetic retina and the reactivity increase with the duration of diabetes(). In early stage (4^{th}- 24^{th} weeks) of diabetes there are reactivity in the glial cells of retina after the induction of streptozotocin [56,60]. Neuronal pathology was associated with activation of both Müller cells and microglia in early induced diabetes in rats (1-4 months)

The consequences of increase in reactive Müller cells on neuronal cell populations in NPDR have been mentioned above and also in our study. At the pre-clinical stage, Müller cells may play conflicting roles in retinal homeostasis, having both protective and cytotoxic effects on retinal neurons. An important feature of Non-proliferative Diabetic Retinopathy that may be related to Müller cell dysfunction and altered neuronal interactions [56,59,60].

6. Summary

In the past, DR has been considered primarily a retinal microvascular disorder but in the present different studies agreed that diabetic retinopathy is a neurovascular disease. Many researches show an early neurodegenerative changes in the diabetic retina, using a variety of experimental methods in detecting the changes using animals in preclinical and human in clinical. In most research the exact cause of the neurodegeneration changes are not studied well but Diabetes-induced uncontrolled levels toxic metabolites, oxidative stress and altered neurotrophicmay be the possible causes of neurodegeneration. Changes that are seen in early DR are reduction in the thickness of different layers of retina, retinal neural cell death, glial reactivity etc.

DR is diagnosed clinically with the onset signs of vascular lesions, but in these different studies the neural changes are the earlier manifestations that intensive research works to be done in the early neural changes in order to help in early diagnosis, protection and treatment of DR.

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