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High-Mobility Group Box-1 Protein a Potential Inflammatory Biomarker in Diabetic Retinopathy

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Abstract

Diabetic retinopathy (DR) is the leading cause of acquired blindness, which is one of the most feared complications of diabetes among young adults. The cause of vision loss in DR is complex and remains incompletely understood. One of the earliest changes in the development of retinopathy is breakdown of blood-retinal barrier (BRB) and the formation of acellular capillaries by unknown mechanism. There is an accumulating body of evidence that demonstrated, chronic low-grade subclinical inflammation and retinal leukocyte stasis are responsible for many of the vascular lesions in DR. In the retina, diabetes induced sustained proinflammatory responses by increasing the production of proinflammatory cytokines, chemokines, and other inflammatory mediators leading to damaged vasculature and neovascularization. An emerging issue in DR research is the focus on the mechanistic link between chronic low-grade inflammation and angiogenesis. Recent evidence has revealed that extracellular high-mobility group box-1 (HMGB1) protein acts as a potent proinflammatory cytokine that triggers inflammation and recruits leukocytes to the site of tissue damage, and exhibits angiogenic effects. The expression of HMGB1 is upregulated in epiretinal membranes and vitreous fluid from patients with proliferative DR and in the diabetic retina. HMGB1 mediates inflammation, breakdown of the BRB, and apoptosis in the diabetic retina. The overall objective of this chapter is to provide the up-to-date literature about the crosstalk between extracellular HMGB1 and DR.

Keywords: Diabetic retinopathy, HMGB-1, Inflammation, Neurodegeneration, Cytokines

1. Introduction

Diabetes is a chronic disease, and it affects more than 230 million people worldwide, and this number is expected to reach 350 million by 2025. Diabetes, a result of body's inability to produce

adequate amounts of insulin or to effectively use the insulin which is required to regulate the amount of sugar in the blood resulting in high levels of circulating blood sugar over a prolonged period. Diabetes is a quickly growing metabolic disorder and fourth of the 10 leading causes of death worldwide, which attributed to one death in every 10 seconds [1]. Several epidemiological studies have recognized that hyperglycemia is a main source of this disease in diabetes. High blood sugar over a prolonged period creates complicated consequences related to the glucose acting as a metabolic substrate and as an intracellular mediator which induces biochemical alteration and finally leads to cells dysfunction. High glucose induces cellular injuries that are known to initiate repair or cell death pathways. During hyperglycemia or altered glucose handling, it promotes nonenzymatic glycation reactions between reducing sugars and the free amino groups on proteins generating advanced glycation end products (AGEs). AGEs formation from protein glycation reactions is thought to be the major pathway involved in the development and progression of different types of complications associated with diabetes, including retinopathy [2]. Glycation-derived free radicals can cause protein fragmentation and oxidation of nucleic acids and lipids. Continuous high level of blood glucose in diabetes damages micro and macro blood vessels throughout the body by altering the endothelial cell lining of the blood vessels which causes more intake of glucose than normal and enhances the level of surface glycoproteins than normal, and results in thicker and weaker basement membrane.

1.1. Diabetic retinopathy

Diabetes threatens vision, and patients with diabetes develop cataracts at an earlier age and are nearly twice as likely to get glaucoma compared to nondiabetic [3]. More than 75% of patients who have had diabetes mellitus for more than 20 years will develop diabetic retinopathy (DR) [4]. According to Wisconsin epidemiologic study of diabetic retinopathy (WESDR), 3.6% of younger-onset patients (type 1 diabetes) and 1.6% of older-onset patients (type 2 diabetes) were legally blind [5]. The retina, a light-sensitive nerve layer that lines the back of the eye, is damaged by hyperglycemia, which is manifested as DR, and is the leading cause of acquired blindness, which is one of the most feared complications of diabetes among young adults. DR is a slow progressive retinal disease and occurs as a consequence of long-standing accumulated functional and structural impairment of the retina by diabetes. It is a multifactorial condition arising from the complex interplay between biochemical and metabolic abnormalities occurring in all cells of the retina. DR has been classically regarded as a microangiopathy of the retina, involving changes in the vascular wall leading to capillary occlusion and thereby retinal ischemia and leakage. And more recently, the neural defects in the retina are also being appreciated [6]. DR is classified into three major categories based on its severity: (i) mild to moderate non-proliferative retinopathy (NPDR), (ii) severe NPDR, and (iii) proliferative retinopathy (PDR). PDR is an advanced form of diabetic eye damage that is caused by chronic hyperglycemia in the blood, which is characterized by the epiretinal outgrowth of fibrovascular membranes at the vitreoretinal interface. The formation of fibrovascular tissue or angiogenesis (i.e., the formation of new blood vessels from existing retinal blood vessels in the vitreous humor) often leads to visual loss due to vitreous hemorrhage and/or tractional retinal detachment. Development of DR is a multifarious process where proteases, growth factors, cytokines, and chemokines such as monocyte chemoattractant

protein-1 (MCP-1), interleukin-8 (IL-8), intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), stromal cell-derived factor-1 α (SDF-1 α), cyclooxygenase-2 (COX-2) and prostaglandin E2 production, vascular endothelial growth factor (VEGF), matrix metalloproteinases (MMPs), connective tissue growth factor (CTGF), and high-mobility group box-1 (HMGB1) protein are released from retinal cells under hyperglycemia and interact with each other as well as activate several signaling pathway to promote neovascularization and fibrosis in retina [7–11]. In this chapter, a major emphasis is given on diabetes-induced HMGB1 protein in the retina, mediates a range of molecules and pathways involved early in the pathophysiology of DR which is briefly discussed and those major cascades of events are shown in the schematic diagram as depicted in **Figure 1**.

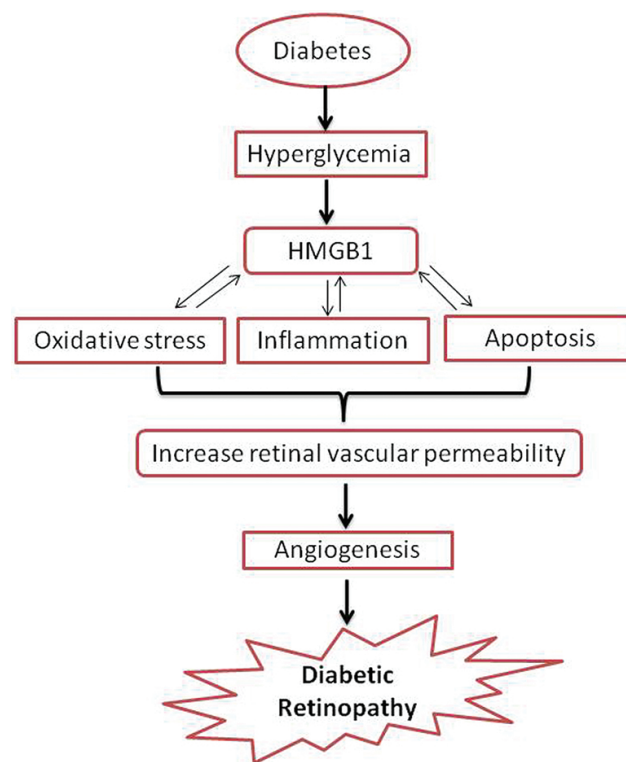


Figure 1. Possible signaling cascade of high mobility group box protein-1 (HMGB1) in diabetic retina. Diabetes causes a rise in HMGB1, which results in increased retinal vascular permeability and angiogenesis through enhancing oxidative stress, apoptosis, and inflammation.

1.2. Inflammation and diabetic retinopathy

Inflammation is a second-line defense process by which the innate immune system of the body guards from infection with foreign pathogen or antigen. The immune system identifies this foreign pathogen or antigen by specific binding receptors, such as receptor for advanced glycation end products (RAGE) and toll-like receptors (TLRs), and activation of these receptors after binding with an antigen induces the production of cytokines (e.g., IL-1 β , IL8, and TNF- α) that further help in the induction or expression of pro-inflammatory mediators [12–14]. As demonstrated by various studies, during the development of DR proinflammatory cytokines,

chemokines and other inflammatory mediators play a central role leading to persistent low-grade inflammation, which influx the leukocytes to the damaged retinal vasculature and induce neovascularization [15, 16]. In the retina or vitreous of diabetic animals and patients, many of the molecular and physiological alterations are found consistent with inflammation, and the gene profile patterns from the diabetic retinas of rodents share resemblance with an inflammatory response [17]. The level of proinflammatory cytokines such as TNF- α , IL-1 β , and IL-6 and chemokines such as MCP-1, interferon- γ -inducible protein of 10 kDa (IP-10), SDF-1, and IL-8 in addition to other key inflammatory proteins including iNOS, COX-2, and MMP-9/gelatinase B, are increased in the vitreous fluid of patients with PDR [18] and in the retina of diabetic animal models [19–21]. Much of the attention on production of proinflammatory cytokines has focused on the IKK-beta/nuclear factor (NF)-kappa (κ)-B pathway, a protein network that enhances transcription of cytokine genes. As inflammation is suggested to be an initiating event of proliferative vitreoretinal disorders, the identification of biomarkers related to leukocyte activities and reflecting the amount of inflammation may provide insight into cellular processes linked to proliferative vitreoretinal disorder progression and would aid in identification of novel targets for therapeutic intervention. Among various cytokines, several recently published studies suggested that HMGB1 protein, a pro-inflammatory cytokine, plays an important role in the development of DR

2. HMGB1

HMGB1 (also known as HMG-1 or amphoterin) is a highly conserved non-histone chromosomal protein which functions as a transcriptional activator in the nucleus [22]. However, HMGB1 is also secreted into the extracellular spaces by a variety of cells involved in immune biology and by necrotic cells. Numerous studies have evaluated proinflammatory cytokine-like activities of extracellular HMGB1 during the event of inflammation [22–25]. The HMGB1 structural functional analyses suggested that the specific post-translational modifications regulate the bioactivity of the molecule.

2.1. HMGB1 structure and functions

The HMGB1 protein is composed of three domains, two positively charged domains at N-terminal (A and B boxes) and one negatively charged (C-terminal). The structure-specific DNA binding is attributed to the A box while the DNA-bending ability is attributed to the B domain. Whereas the C-terminal (acidic tail) of this protein helps in maintaining the stability and DNA bend and not DNA binding. The A box and B domain are responsible for the specific DNA-binding and DNA-bending ability, respectively, whereas the C-terminal maintains the protein stability [26]. In addition, the primary structure consists of three oxidation-sensitive unpaired cysteine residues, C23, C45, and C106, which facilitates the activities of extracellular HMGB1. During the event of inflammation, HMGB1 exerts its cytokine-like activities, the unpaired C106-residue-containing thiol group is essential for the interaction with TLR4 to generate cytokine-inducing capacity. The inactive form of HMGB1 contains terminally oxidized cysteine residues C23, C45, and C106, causing resolution of inflammation. The chemoattractic

activities of HMGB1 are correspond to a fully reduced form all three cysteine residues C23, C45 and C106. Recently, a great deal of research evidence indicated the major role of HMGB1 in the regulation of oxidative stress, apoptosis, inflammation, and angiogenesis in various diseases. HMGB1 binds with multiple receptors including RAGE and TLR2, TLR4, and TLR9. Signaling through these receptors leads to activate various cellular signaling pathways which induce proinflammatory cytokines and chemokines and escalate leukocyte adhesion in various diseases. HMGB1 is not just released in reply to inflammatory stimuli, but itself facilitates the production of inflammatory mediators [27, 28]. In **Figure 2**, the molecular docking shows the interaction between HMGB1 and RAGE. All of this explains the implication of HMGB1 in mediating fundamental cellular events such as transcription, recombination, replication, and tissue damage repair and plays a role in inflammatory reaction through its cytokine-like activities.

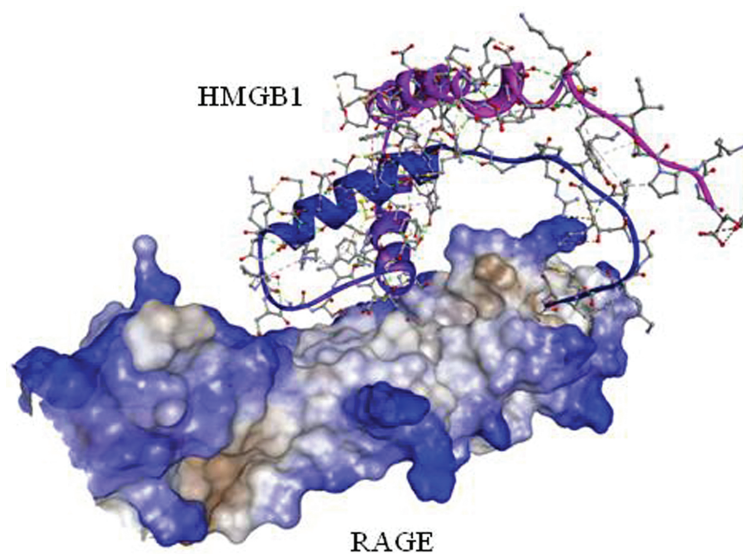


Figure 2. The binded form of HMGB1 and receptor for advanced glycation endproducts (RAGE). The most stable bound conformation obtained after the protein-protein docking by using the ZDOCK.

2.2. HMGB1 and inflammation

Inflammation is a cascade of biochemical immune responses involving soluble factors, vascular permeability, and leukocyte migration in response to pathophysiology associated to acute or chronic inflammatory conditions. Various leukocytes, which normally reside in blood, must move into the inflamed tissue via extravasation to aid in initiation and maintenance of inflammation. In an event of immune response, HMGB1 is actively secreted from the nucleus to the extracellular space by different leukocytes and endothelial cells and functions as a proinflammatory cytokine. In the culture media of lipopolysaccharide (LPS) -treated cell, HMGB1 could be detected after 8 hours and highest at 18 hours. Similarly, HMGB1 could be released from serum samples after 8–32 hours mice challenged with LPS or TNF- α . In addition, recombinant HMGB1 injected intraperitoneally was lethal to LPS-sensitive or LPS-resistant

mice. Thus, extracellular HMGB1 seems to have a remarkable proinflammatory effect. Leukocyte transmigration or extravasation is the movement of leukocytes out of the circulatory system, toward the site of tissue damage, or infection is mediated by cascade sequence of adhesion and activation events that ends with extravasation of the leukocyte, whereby the cell exerts its effects on the inflamed site. Extracellular HMGB1 proteins promote the recruitment of leukocytes across endothelial barriers through their effects on integrin signaling mainly involving RAGE, a primary receptors for HMGB1 to mediate chemotaxis, proliferation, and differentiation activities [23, 29]. The receptor-ligand interactions involved in this complex process is mediated through adhesive interactions between leukocytes and endothelial cells. In the time of inflammation, the adhesive glycoproteins expressed on the surface of both leukocytes (CD11/CD18) and endothelial cells ICAM, endothelial-leukocyte adhesion molecule (ELAM) interact and facilitate the leukocyte adherence [30]. In recent years, several published reports have indicated that HMGB1, which is released in an immune response by a variety of cells, involved in immune biology and by necrotic cells, also mediates the leukocyte adherence by inducing inflammatory cytokines [23, 25, 31, 32]. Various studies have suggested that binding of secreted HMGB1 to its receptors activates various signaling pathways leading to activation of transcription factor that induces production of adhesion molecules, cytokines, and chemokines [33–39]. However, the exact mechanism by which HMGB1 mediates leukocyte adhesion has not been defined, but the one possible mechanism can be thought is that extracellularly secreted HMGB1 neutralizes or inactivates the endothelial cell-derived antiadhesive substance. In addition to leukocyte adhesion, recent studies also documented that involvement of extracellular HMGB1 in the disruption of vascular barriers such as breakdown of the blood-brain barrier (BBB) and blood-retinal barrier (BRB). Major cascades of events are shown in the schematic diagram as depicted in **Figure 3**.

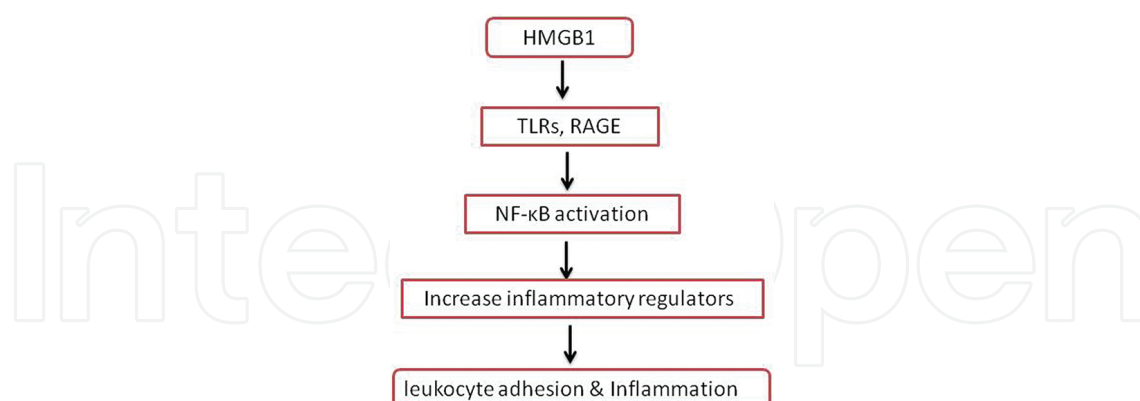


Figure 3. Hypothetic diagram of HMGB1-mediated leukocyte adhesion and inflammation in diabetic retina via RAGE or TLR.

2.3. HMGB1 as a biomarker in the diseases

The biomarkers are of growing importance in the difficult fields of predict, diagnose, and therapy monitoring in various disease. HMGB1 is one potential biomarker for inflammatory

and autoimmune-related diseases, as established by both animal models and clinical studies. In animal models, HMGB1 expression significantly increases and is associated with immune modulation, inflammation, angiogenesis, trauma, cardiac dysfunction, diabetic complications, and metastasis. Clinical studies have also established a relationship between HMGB1 and in immune modulation in many acute and chronic diseases such as trauma, acute inflammation, and autoimmune diseases, making HMGB1 a viable candidate to add to the multiple biomarker lists. Extracellular release of HMGB1 from immunological active cells or necrotic cell plays an essential role in autoimmune diseases like rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE), as well as in acute inflammatory events like sepsis and trauma. It was demonstrated that increased levels of HMGB1 in the serum samples from patients with SLE correlated positively with disease activity measured by SLE disease activity index scores and proteinuria, as well as with levels of anti-HMGB1 antibodies. In addition, the presence of HMGB1-specific antibodies suggests a pathogenetic role of HMGB1/anti-HMGB1 immune complexes in SLE [40]. RA is characterized by enhanced angiogenesis, and HMGB1 was shown to promote angiogenesis in RA by enhanced expression of VEGF and HIF-1 α activation [41]. Furthermore, HMGB1-induced angiogenesis was inhibited by cilostazol via SIRT1 activation in synovial fibroblasts from RA [42]. Several studies have shown that HMGB1 levels were elevated in all kinds of trauma such as severe trauma, burn trauma, or iatrogenic trauma [43–46] and suggested that this elevated level of HMGB1 derived from trauma-induced cell death. Anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitides (AAV) are systemic inflammatory disorders in which the serum level of HMGB1 did not change significantly and remains same level as control suggesting HMGB1 is not a useful biomarker in AAV [47]. Viral hepatitis E clinically ranges between acute self-limiting hepatitis (AVH) and acute liver failure (ALF) in which the mean circulating HMGB1 levels were significantly higher in ALF than AVH [48]. In cancer, HMGB1 regulates the transcription of many cancer genes, such as *E-selectin*, *TNF- α* , *insulin receptor*, and *BRCA1* [49–51]. In the serum samples of colorectal carcinoma patients, the HMGB1 level was increased compared to those in healthy controls [52]. In malignant cancer, HMGB1 play an important role in creating a pro-inflammatory microenvironment which resulted in promoting tumor growth, angiogenesis, and metastasis

3. HMGB1 and diabetic retinopathy

Extensive experimental data generated in both tissue culture and animal models as well as clinical indicated that the progression of DR development involved alteration in the vascular wall leading to capillary occlusion and promoting ischemia and leakage in the retina. And newly, retinal neurodegeneration is also being greatly appreciated [6]. Many recent research are focused on retinal vascular dysfunction, such as breakdown of the BRB, altered tight junctions, death of capillary cells, and thickening of basement membrane. One of the early pathological sign of retinopathy in experimental diabetes models include increased retinal permeability. The possible mechanism by which diabetes-induced retinal permeability is considering the inflammatory reaction between reactive oxygen species (ROS) and cell-adhesion molecules resulted in breakdown of the BRB and loss of endothelial cells by leukocyte

adhesion [53, 54]. The earlier events in diabetic retinal inflammation are the adhesion of leukocytes to the microvasculature which encourages the induction of adhesion molecules such as ICAM-1 and P-selectin, on the endothelium and its leukocyte counter-receptor CD18 [54]. Occludin is a transmembrane protein located at the tight junction which confers the cell-to-cell adhesion and interaction [55]. A high level of occludin was shown to maintain endothelial cells BRB, occludin expression is specific to vascular endothelial cells with strong barrier properties and its down-regulation increases vascular permeability [55–57]. Diabetes increases retinal vascular permeability and reduces the level of occludin in retina; similarly, administration of HMGB1 to normal eye enhances the vascular permeability and lowers the expression of occludin. In addition, retinal endothelial cell exposed to HMGB1 reduces the trans-endothelial electrical resistance [58]. Furthermore, one essential consequence of the inflammatory process by which vascular permeability enhances is the induction of adhesion molecules such as ICAM-1 by leukocyte endothelial cell interaction. The expression of ICAM-1 increases in the diabetic retina and its expression significantly correlated with retinal vascular permeability. Intravitreal injection HMGB1 induces up-regulation of ICAM and blocker of HMGB1 attenuates diabetes-induced ICAM-1 expression in the retina [58]. However, the detailed molecular mechanisms by which HMGB1, leukocyte adhesion, and vascular permeability cross-talk in the development of DR is not discussed much and it needs to be investigated further.

AGEs are key mediators of almost all complications associated with diabetes [59, 60]; hyperglycemic environment facilitates ROS generation which promotes AGEs formation. AGEs damages microvascular and macrovascular by promoting crosslink formation between proteins, which alters their structure and function, as in cellular matrix, basement membranes, and vessel-wall components. In addition, AGEs interact with a variety of cell-surface AGE-binding receptors, leading either to their endocytosis and degradation or to cellular activation and pro-oxidant, pro-inflammatory events. AGEs binding to RAGE induces ROS generation, which in turn activates the pleiotropic transcription signaling factor such as NF- κ B, inducing multiple pathological responses [61]. In the diabetic retina, AGEs formation contributes to enhance angiogenesis in retinal microvessels, and endothelial cell incubated with AGE-BSA enhances the production of proangiogenic factor such as VEGF and increases cell proliferation and tube formation through NF- κ B activation pathways [62, 63]. Therefore, the contribution of AGEs in regulation a pro-angiogenic factor to enhance angiogenesis in the development of DR cannot be ignored. In fact, epiretinal membranes obtained from PDR patient were shown the expression of AGE, RAGE, and HMGB1 by vascular endothelial cells and stromal cells and its presence correlated with level of vascularization [64]. In the retina of experimental diabetic animals, increased expressions of AGEs, RAGE, and HMGB1 have been documented [11, 65]. In addition, the level of HMGB1, AGE, and RAGE is upregulated and significantly correlated with biomarkers of inflammation in the vitreous of patients with PDR [11, 57, 66]. RAGE is a cell surface receptor that binds AGEs, which are expressed by the endothelial cells that form the inner lining of blood vessels [67]. In the diabetic retina, HMGB1 and RAGE upregulate and interact with each other and activate the extracellular signal-regulated kinase 1 and 2 (ERK1/2) phosphorylation [58], leading to induction of pro-angiogenic molecules such as VEGF, TNF, and IL-8 [11, 68, 69]. In diabetes, HMGB1 up-regulation is associated with microvessel and

macrovessel abnormalities, in the eye it damages retinal function, and its inhibition may be able to improve this retinal defect caused by hyperglycemia [11, 25, 69].

Inflammation all known to cause retinal cells death largely occurs via apoptosis in diabetes and the possible signaling mechanism involved in this is believed to be mediated by activation of NF- κ B pathways. In the retina of diabetic animal and retinal capillary cells incubated with high glucose, NF- κ B is activated and this causes production of inflammatory cytokines and apoptosis [70]. Thus, activation of NF- κ B in the retina is considered as pro-apoptotic factor and its activation is considered as a negative regulator of cell survival [71, 72]. However, in various cancers, activation of NF- κ B is considered as an anti-apoptotic factor and positive regulator of cell survival, thus suggesting a differential role of activation of NF- κ B in diseases. HMGB1 has been shown that in diabetic retina induces NF- κ B activation [58], and recently, it was shown that HMGB1 directly mediates retinal endothelial apoptosis [32]. The possible mechanism by which HMGB1 induces retinal cell death in diabetes may be mediated by ROS via NF- κ B activation because it is known that ROS activated NF- κ B and induced apoptosis retina. Various studies have drawn a connection between oxidative stress and apoptosis, and in retina, diabetes-induced ROS modulates or activates pro-apoptotic mediators has been shown [1, 68, 72, 73]. Supplementation of antioxidants to diabetic rats prevents the retina from oxidative stress and apoptosis, and also the development of retinopathy [1, 74]. In addition, it was shown that local oxidative stress that has a neurodegenerative influence in the diabetic retina is prevented by constant intake of an antioxidant-supplemented diet such as lutein [75]. Recently, various clinical investigators detect neuronal dysfunction at very early stages of diabetes and numerous abnormalities in the retina can be identified even before the vascular pathology appears [76, 77], thus suggesting a direct effect of diabetes on the neural retina. In streptozotocin (STZ)-induced diabetic mice, capillary lesion occurs at very early stages of DR, which resulted in loss of inner retinal neurons by increasing apoptosis [78]. At the early stage of diabetes, retinal ganglion cell dies by apoptosis, which is necessary to maintain the normal function of retina [79]. The mechanisms by which diabetes induce retinal neurodegeneration involve metabolic stress, altering the regulation of many growth factors which are involved in the process of neuronal death. Recently, it was demonstrated that HMGB1 is the main mediator bridging persistent neuroinflammation and chronic progressive dopaminergic neurodegeneration in neurodegenerative diseases, such as Parkinson's disease [80]. It was also reported that release of HMGB1 to extracellular space arbitrates to postischemic brain and retina damage and that blocking of HMGB1 prevents postischemic neurodegeneration [81]. Neurotrophins are a family of protein which regulates many aspects of neural function, its survival and developments, and it is sensitive toward oxidative stress. Brain-derived neurotrophic factor (BDNF), a protein belonging to the neurotrophin family, is expressed in retinal cells such as ganglion cells and Müller cells [82, 83] and is essential for its development, survival, and its synaptic activity [84]. In retina, diabetes induces retinal neuropathy by reducing the expression of BDNF and can be ameliorated by an exogenous administration [75]. Various study suggested that in the diabetic retina the BDNF levels, and synaptophysin, a synaptic vesicle protein for neurotransmitter is reduced by ROS [73, 85, 86]. Glutamate, the excitatory neurotransmitter in the retina, mediates the transfer of visual signals from the retina to the brain by photoreceptors, bipolar cells, and ganglion cells. Excitotoxicity is a state of high glutamate level,

damage to the retinal ganglion cell by activation of ionotropic and metabotropic glutamate receptors [87, 88]. Glutamine synthetase, an enzyme which converts glutamate to glutamine, significantly decreased in the diabetic rat retinas, which resulted in elevated glutamate levels in the diabetic retinas, which might induce retinal neurodegeneration via glutamate excitotoxicity [87, 89]. Synaptophysin protein is decreased in the retina of the STZ-induced diabetes model through the ROS-ERK_{1/2} and suggested the cross-talk between mitogen-activated protein kinases (MAPK) pathway signals and neurodegeneration [75, 90]. Recently, Abu El-Asrar et al. studied the involvement of HMGB1 in retinal neurodegeneration and showed that diabetes and intravitreal administration of HMGB1 induces up-regulation of lipid peroxidation and cleaved caspase-3 and glutamate, whereas BDNF, synaptophysin, tyrosine hydroxylase, glutamine synthetase, and glyoxalase 1 were downregulated in the retinas and inhibitor of HMGB1 attenuates diabetes which induced these changes [85, 91], and they suggested that the early retinal neuropathy induced by diabetes involves HMGB1 and can be ameliorated by inhibition of HMGB1. Thus, targeting the HMGB1-mediated signaling cascade may constitute a new beneficial approach to inhibiting the progress of DR.

4. Future perspective of HMGB1 as a biomarker for diabetic retinopathy

Ischemia-induced angiogenesis is the pathological hallmark in PDR and remains a significant cause of vision loss due to vitreous hemorrhage and/or traction retinal detachment. Therapeutic regulation of angiogenesis has emerged as an attractive approach for the treatment of PDR. Recently, extracellular HMGB-1 has been recognized as a potent proinflammatory cytokine that triggers inflammation and recruits leukocytes to the site of tissue damage [11, 23, 25, 92], and exhibits angiogenic effects. The angiogenic potency of HMGB1 has been confirmed in several *in vitro* and *in vivo* model systems providing a strong clinical evidence for the proangiogenic function of HMGB1 [11, 41, 42, 69, 92]. It was demonstrated that HMGB1 localized in vascular endothelial cells and stromal cells in epiretinal fibrovascular membranes from patients with PDR. In addition, increased levels of HMGB1 positively correlated with the levels of the inflammatory biomarkers such as ICAM-1 in the vitreous fluid of PDR patients [11, 64]. Furthermore, intravitreal injection of HMGB1 induces the expression of RAGE, activated ERK1/2 and activated NF- κ B in the retinas of rats [58]. VEGF and HIF-1 α is the major angiogenic factor in PDR that promotes neovascularization and vascular leakage. HMGB1 was shown to enhance the expression of VEGF and HIF-1 α in retinal endothelial cell and in the retina [69]. Retinal leukostasis and leukocyte adhesion to the retinal microvasculature is associated with endothelial cell death, capillary occlusion, and increased vascular permeability, which all contribute to the progression of DR [15, 16, 18, 93]. Administration of HMGB-1 in the vitreous of normal rats has been shown to enhance the retinal vascular permeability, and retinal endothelial cell treated with HMGB1 causes reduction in transendothelial electric resistance [58]. Recently, a great deal of evidence has indicated that diabetes induced ROS generation implicated in the developmental DR and it is considered as important mediator of apoptosis in retina. In diabetic microenvironment, retina and capillary cells experience increased oxidative damage and the therapy which blocks oxidative stress also blocks the

progression of retinopathy [1, 72, 74, 75]. A strong relation between oxidative stress and accelerated retinal capillary cells death was observed in the pathogenesis of DR [72, 74]. HMGB1 levels and oxidative stress marker protein carbonyl content levels were significantly correlated in the vitreous fluid of PDR patients [68]. In addition, HMGB1 treatment enhanced ROS generation and up-regulation of retinal apoptotic markers such as poly (ADP-ribose) polymerase (PARP)-1 and cleaved caspase-3 production by human retinal endothelial cells [68]. Similarly, administration of HMGB1 in the vitreous of normal rats increases ROS production and markers of apoptosis [68]. Furthermore, it was demonstrated that the HMGB1 mediates pericyte death via cytotoxic activity of glial cells, whereas it directly induces endothelial cells death and regulates endothelial cell activity [32]. Therefore, HMGB1 may be a potential biomarker because it exerts a multitude of functions in the development of DR.

5. Conclusions

As described in this chapter, extensive research progress has been made in investigating the pathophysiology of the disease; however, because early stages of DR is scarcely explored and much of the ophthalmic therapy for DR is focused on severe stages (pre-proliferative stage) of the disease, the exact molecular mechanism has not been elucidated. Here, we describe the potential role of HMGB1 in DR as a result of its pro-inflammatory properties and multiple activities. Because HMGB1 destabilizes the markers of oxidative stress, apoptosis and inflammation in the retina from hyperglycemia, it suggests that early blockade of HMGB1 may be an effective strategy to prevent the progression of DR.

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