Pathology in Diabetic Retinopathy

Subjects: Allergy

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Diabetic retinopathy (DR) as a microangiopathy is the most common complication in patients with diabetes mellitus (DM) and remains the leading cause of blindness among adult population.

Keywords: diabetes ; inflammation ; retinopathy

1. Introduction

Diabetes mellitus (DM) is a group of metabolic disorders characterized by a high blood glucose level (hyperglycemia) over a prolonged period [1][2]. Classification of diabetes in clinical work includes a division into type 1 diabetes and type 2 diabetes. It varies in degree of heterogeneity in clinical manifestations, accurate diagnosis, comorbidities, and treatment [3][4][5]. Diabetes can cause many health complications both acute (e.g., diabetic ketoacidosis, hyperosmolar hyperglycemic state) and long-term (e.g., cardiovascular disease, stroke, chronic kidney disease, foot ulcers, and damage to the nerves or to the eyes) [6][7][8]. Diabetic retinopathy (DR) is a major microvascular complication of diabetes mellitus and remains the leading cause of visual loss in the adult population. It is estimated that diabetes might affect up to 34% of the worldwide population aged 40 and older by 2035 [9][10]—according to this data, DR is growing into a worldwide health problem as well. A study by Lin et al. [11] revealed that women with DM type 2 had a higher prevalence of diabetic retinopathy than men, but men suffered from more severe retinopathy, poor vision, or blindness. DR impacts not only the quality of life, but also predicted vascular and non-cancer mortality, prolonged QT interval, or life-threatening arrhythmia [12][13]. Considering clinical manifestations of vascular abnormalities in the retina, DR is divided into two stages: non-proliferative diabetic retinopathy (NPDR) and proliferative diabetic retinopathy (PDR). In the initial stage of NPDR, hyperglycemia and altered metabolic pathways lead to oxidative stress, leakage of multiple inflammatory cytokines and plasma proteins and then to the development of neurodegeneration, disruption of the blood-retinal barrier (BRB) and progressive retinal pathologies. Early hallmarks of NPDR, detected under fundus photography include increased vascular permeability, capillary occlusion, microaneurysms, dot intraretinal hemorrhage and hard exudates. As disease progresses, a more advanced stage of DR turns uncontrollably into PDR where severe hypoxia leads to neovascularization, vitreous hemorrhage, and retinal detachment such as traction retinal detachments (TRDs) and combined traction/rhegmatogenous retinal detachments (TRD/RRDs) which remains the most common reason for vitrectomy in patients with proliferative diabetic retinopathy [14][15]. There are four stages of diabetic retinopathy [16][17][18]:

- Mild non-proliferative diabetic retinopathy (NPDR)—there may be no symptoms in this stage; microaneurysms develop on the tiny vessels in the retina, the light-sensitive back layer of the eyeball; leak fluid into the retina might be present.
- Moderate NPDR—more vessels become weak and blocked; they begin to be swollen and distorted in size and lose their ability to properly transport blood.
- Severe NPDR—more blood vessels become blocked which disrupts blood supply to areas in the retina with compensation by signaling the retina to grow up new blood vessels.
- Proliferative diabetic retinopathy (PDR)—the most advanced stage of retinopathy where new, weak, and inefficient blood vessels grow along the inside surface of the retina and into the vitreous gel; they are more likely to leak and bleed causing retinal detachment.

The classification of DR according to ophthalmoscopic features is shown in **Table 1**.

Table 1. Classification of diabetic retinopathy—according with [18].

Type	Ophthalmoscopic Features
Mild NPDR	Microaneurysms

Type	Ophthalmoscopic Features
Moderate NPDR	At least two of the following features: * Microaneurysms * Retinal hemorrhages * Hard exudates
Severe NPDR	Any one of the following features: * 20 hemorrhages in each of the four quadrants * Venous beading in two quadrants * IrMAs in one quadrant
PDR	At least one of the following features: * Neovascularization * Vitreous hemorrhage

NPDR—non-proliferative diabetic retinopathy; PDR—proliferative diabetic retinopathy; IrMAs—Intraretinal microvascular abnormalities.

The distortion of visual images, decrease in visual acuity and vision loss in patients with DM can occur at any stage of DR. The most common cause of vision loss in patients with DR is diabetic macular edema (DME) which is the result of swelling or thickening of the macula due to sub- and intra-retinal accumulation of fluid in the macula triggered by the breakdown of the BRB. Currently the mainstay of therapy for DR aim at managing the microvascular complications, including intravitreal administration of pharmacological agents with steroids as one possible option, along with laser photocoagulation and vitreoretinal surgery [11][17][19].

2. Pathology in DR

Hyperglycemia is considered as an important factor in the pathogenesis of retinal microvascular damage which leads to DR. Metabolic pathways that are considered during hyperglycemia-induced vascular damage including advanced glycation end products (AGEs) accumulation, the protein kinase C (PKC) pathway, and the polyol and the hexosamine pathway. Additionally, dilation of blood vessels and blood flow changes are the earliest responses to hyperglycemia from retinal blood vessels in diabetic patients. Other hallmarks of the early events of DR are pericytes loss triggered by high glucose concentration with the following outpouching of capillary walls and microaneurysm formation, apoptosis of endothelial cells, and thickening of the basement membrane. Next, pronounced loss of pericytes and endothelial cells collectively contributes to the impairment of the BRB and results in capillary occlusion and ischemia. Retinal ischemia/hypoxia leads to activation of hypoxia-inducible factor 1 (HIF-1) and upregulation of angiogenic factors such as angiopoietins (Ang-1, Ang-2) and most vascular endothelial growth factor (VEGF) [9][20].

2.1. DR—An Inflammatory Disease

Originally, DR was considered a purely microvascular disease. Currently, chronic, low-grade inflammation plays a key role in the pathogenesis of DR that leads to changes in the retinal microcirculation and was detected widely in different stages of DR in both diabetic animal models and in the retinas of diabetic patients. This pathology affects neuronal and vascular components of the retina and what is more, it shows some similarities with chronic inflammatory diseases like infiltration of inflammatory cells, expression of different effectors such as cytokines responsible for damage to the retina, edema, neovascularization, or destruction of tissues.

2.1.1. Role of Inflammatory Cells in DR

Leukostasis is an occlusion of retinal microvasculature by monocytes, macrophages and granulocytes and was reported in an animal model of DM and in the early stage of DR in patients [21][22][23]. Additionally, increased leukostasis might be correlated with endothelium damage and BRB impairment through the Fas (CD95)/Fas-ligand pathway. Leukocyte-endothelium adhesion occurs according to upregulation of leukocyte b2-integrins (CD11a, CD11b, CD18) and expression of endothelial cell adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1) and selectins (E-selectin). Additionally, the plasma expression of VCAM-1 and E-selectin is correlated with the severity of DR. Retinal glial cells, consisted of astrocytes, Müller cells and microglia, are responsible for structural support and maintaining homeostasis in the retina. Nevertheless, in the condition of hyperglycemia and oxidative stress, glial cells are dysfunctional and they enhance the production of proinflammatory cytokines (TNF-α, growth factors, IL-1β, IL-6) which are involved in the onset and amplification of inflammation in the diabetic retina. In addition, the secretion of proinflammatory cytokines by glial cells plays a role in the infiltration of monocytes and T lymphocytes and on the other hand chronic inflammation induces fibrotic processes which induce scar formation and then retinal detachment [24][25].

2.1.2. Role of Inflammatory Chemokines in DR

Some chemokines have been shown to be involved in the pathogenesis of DR. In some recent studies, monocyte chemotactic protein-1 (MCP-1) and macrophage inflammatory protein-1 alpha (MIP-1a) have been reported to be elevated in diabetic patients [14][26][27]. Moreover, a level of other inflammatory cytokines such as interleukin 1 (IL-1), IL-6, IL-8, and tumor necrosis factor alpha (TNF-α) was upregulated in diabetic patients with DR. Additionally, there is a correlation between the presence of high levels of growth factors and inflammatory cytokines in the eye fluids and inflammation in patients with DR. In the retina, under hyperglycemic stress, microglia is activated, which leads to the upregulation of the Nuclear Factor kappa-light-chain-enhancer of activated B cells (NF-κB) followed by increase in oxidative stress with induction of pro-inflammatory cytokines, such as IL-1b, VEGF and TNF-α, chemokines, and adhesion molecules (E-selectin, ICAM-1). Above-described activation of the inflammatory process in DR causes the increase in vascular permeability, loss of pericytes, and the appearance of microaneurysms. Because the retina uses high quantities of glucose and oxygen to generate energy by using the mitochondrial electron transport chain (ETC), reactive oxygen species (ROS) and free electrons are increased during inflammatory process in DR which causes release into the cytosol harmful lipids, proteins and oxidized mitochondrial DNA (mtDNA). They are recognized as damage-associated molecular profiles (DAMP) by the Toll-like receptors TLR4, TLR9 and NLRP3, which in turn enhances the production and activation of pro-IL-1β and pro-caspase-1 [26][28].

2.2. VEGF

“Vascular Permeability Factor” (VPF) was described by Senger et al. in 1983 [29], then Ferrara and Henzel in 1989 [30] discovered its mitotic effect on endothelial cells and proposed the name vascular endothelial growth factor (VEGF). Nowadays, “a family” of vascular endothelial growth factors consists of several members: VEGF-A (called generally VEGF, the prototype molecule of a family, discovered first); VEGF-B, VEGF-C, and VEGF-D (also known as c-Fos-induced growth factor, FIGF); placenta growth factor (PlGF); and the viral VEGF-E encoded by strains D1701, NZ2 and NZ7 of the parapoxvirus Orf (which causes pustular dermatitis). VEGF itself is a heparin-binding, homodimer glycoprotein; its weight is 46 kDa, with a different number of amino-acids that are produced in human cells by alternative splicing (for VEGF-A, VEGF-B, and PGF) and processing (VEGF-A, VEGF-C, and VEGF-D). VEGF gene expression is physiologically regulated by oxygen tension—in hypoxia condition, the transcription factor HIF-1 (hypoxia-inducible transcription factor 1) binds to the hypoxia-responsive enhancer elements (HREs) at VEGF gene affecting transcriptional upregulation [31][32][33]. Moreover, some growth factors and cytokines, including tumor growth factor (TGF), basic fibroblast growth factor (FGF-2), interleukin-1 and interleukin-6 (IL-1, IL-6) can act synergistically with hypoxia [34]. VEGF made up of 121 and 165 amino acids and is produced mainly by neutrophils, platelets, endothelial cells, fibroblasts, epithelial cells, and macrophages in a soluble and freely diffusible form, whereas VEGF consisted of 189 and 206 amino acids is associated with cells’ surface. VEGF acts biologically by receptors tyrosine kinases (RTKs). These receptors have three parts: an extracellular immunoglobulins-like domain, a middle part located in the thickness of the cell membrane, and an intra-cytoplasmic part. VEGF binds to trans-membrane tyrosine kinase receptors, inducing their dimerization and transphosphorylation. VEGF-A binds to VEGFR2 (also called KDR/Flk-1) and VEGFR1 (Flt-1), VEGF-C and VEGF-D bind VEGFR2 and VEGFR3 (Flt4), PlGF and VEGF-B bind only to VEGFR1, and VEGF-E binds only to VEGFR2. VEGFRs differ considerably in signaling properties and are expressed by endothelial cells, epithelial cells, or activated macrophages. In addition, it was found that Neuropilin-1, a trans-membrane protein lacking tyrosine kinase activity, acts as a co-receptor for VEGF-A [35][36]. VEGF plays a key role in the process of vasculogenesis (the de novo formation of the embryonic circulatory system) and angiogenesis (the growth of blood vessels from pre-existing vasculature) in physiological conditions, i.e., during post-natal and skeletal growth, reproductive functions, embryogenesis with endothelial cells’ growth, menstrual cycle, and wound healing. On the other hand, VEGF has also been implicated in pathological angiogenesis, e.g., in cancers and metastasis, retinal neovascularization during DR, age-related macular degeneration, chronic obstructive pulmonary disease, asthma, ischemic heart disease, and rheumatoid or autoimmune diseases [37][38]. Drugs such as aflibercept (binds to circulating VEGF and acts like a “VEGF trap”), bevacizumab and ranibizumab (recombinant humanized monoclonal antibodies that blocks angiogenesis by inhibiting VEGF), or pegaptanib (a pegylated anti-VEGF aptamer, a single strand of nucleic acid that binds to the 165 isoform of VEGF) can inhibit VEGF and control or slow some of those diseases [39][40].

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