NEW INSIGHTS INTO THE PATHOGENESIS OF DIABETIC RETINOPATHY*

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ABSTRACT

Diabetic retinopathy (DR) and diabetic macular edema (DME) are, and will continue to be (because of the increasing prevalence of diabetes), leading causes of blindness in the working-aged population of most developed countries. Although laser photocoagulation is an effective treatment for DR, reducing the overall risk of moderate visual loss by up to 50%, ocular complications of diabetes remain a major health challenge, due in part to insufficient screening for both diabetes and its ocular complications, as well as our incomplete understanding of the complex pathophysiologic processes involved in diabetes-related ocular complications. In recent years, vascular endothelial growth factor has been investigated and recognized as one of the key mediators of vascular hyperpermeability and neovascularization. This article summarizes our understanding of DR and DME pathophysiology, and discusses a proposed new classification scheme that may be of more practical benefit for ophthalmologists treating patients with diabetes. (Adv Stud Ophthalmol. 2008;5(1):16-21)

D iabetic retinopathy (DR) and diabetic macular edema (DME) are leading causes of blindness in the working-aged population of most developed countries.¹ The increasing number of individuals with diabetes mellitus worldwide suggests that DR and DME will continue to be a major contributor to visual loss in the years to come.

Two landmark clinical trials have demonstrated that effective treatment (laser photocoagulation) for DR could reduce severe vision loss by 50%; the Early Treatment Diabetic Retinopathy Study (ETDRS) and the Diabetic Retinopathy Study (DRS).² ³ In both studies, patients with proliferative DR (PDR) or non-proliferative DR were randomly assigned to laser photocoagulation.³ ⁴ The treated eyes showed significantly reduced progression to PDR (DRS) and moderate visual loss (ETDRS).² ³ Nonetheless, ocular complications of diabetes remain a major health challenge, perhaps due in part to insufficient screening for both diabetes and its ocular complications. However, better understanding of the mechanisms involved in the pathogenesis of diabetic complications, combined with careful follow-up and prompt intervention when necessary, could reduce potential visual disabilities.

THE PATHOGENESIS OF OCULAR COMPLICATIONS

The pathogenesis of diabetes-related ocular complications is complex and probably not yet completely understood. In recent years, vascular endothelial growth factor (VEGF) has been investigated and recognized as one of the key mediators of vascular hyperpermeability and neovascularization.

Our current understanding of the metabolic pathways contributing to the development of diabetic microvascular complications is summarized in the Figure. Briefly, hyperglycemia is responsible for 3 important pathogenetic mechanisms—the creation of
advanced glycation end products (AGE), increasing diacylglycerol, and increasing the oxidative stress—all of which are responsible for an increase in protein kinase C (PKC) and therefore, other by-products of activity of cellular second-messenger systems, such as altered gene expression and protein functions. AGEs are molecules formed from the nonenzymatic reaction of reducing sugars with free amino groups of proteins, lipids, and nucleic acids. These initial products (ie, AGE precursors) are reversible, depending, in part, on the concentration of glucose. High glucose concentrations will cause irreversible formation of AGEs, which inflict cellular damage because they are able to crosslink to other proteins (eg, basement membrane proteins, cellular matrix proteins, and vessel-wall components), altering the proteins’ function. In addition, AGEs can bind to some receptors, causing their reuptake into the cell (and subsequent degradation) or initiating cellular activation and oxidative, inflammatory events. PKC also stimulates the production of insulin-like growth factor, which is associated with microvascular damage and, in an animal model of diabetes, breakdown of the blood-retinal barrier. Subsequent to, or perhaps concurrent with, PKC activation is the stimulation of VEGF, which is important for angiogenesis, in addition to increased vascular permeability. Importantly, VEGF is an endogenous compound that is inherently beneficial. Its effects are normally counterbalanced by antiangiogenic factors. Diabetic conditions destabilize the balance between these forces, which clears the way for initiation of neovascularization.

**UNDERSTANDING THE ROLE OF VEGF IN DR**

A full review of VEGF is beyond the scope of this article. However, it has been reviewed elsewhere. VEGF is, in fact, VEGF-A, 1 of 7 in the VEGF family of proteins that are involved in angiogenesis, lymphangiogenesis, and vascular permeability. VEGF-A is the best understood protein in this family and is often simply referred to as VEGF. The gene for VEGF-A resides on chromosome 6 and creates 9 isoforms, depending on how it is spliced. The isoforms vary based on the number of amino acids and their heparin binding affinity (which can also result in cell binding with cell-surface– and basement-membrane–associated heparan sulfate proteoglycans) and are expressed in a wide variety of tissues. VEGF-A is a dimeric glycoprotein that can bind to 2 different tyrosine kinase receptors, which are located primarily on vascular endothelial cells. A third type of receptor binds to a limited number of VEGF-A isoforms and is found on not only endothelial cells but also neural tumor cells. In the eye, VEGF and its receptors are expressed in physiologic conditions (particularly in the retinal pigment epithelium [RPE]). In fact, the primary sources of VEGF are neural cells, including the ganglion, Müller cells, and RPE cells. VEGF is trophic for the choriocapillaris and is required for the maintenance of the choriocapillaris fenestrae.

In vitro and in vivo (animal and human) studies have shown that VEGF plays a role in several physiologic conditions, namely embryogenesis, ovulation, bone growth, wound healing, and formation of vascular collaterals in ischemic heart and limbs. VEGF is also known to play a key role in pathologic conditions. For example, VEGF messenger RNA (mRNA) is expressed in the vast majority of human tumors, and VEGF blockade with anti-VEGF antibodies decreases
tumor perfusion, vascular volume, and interstitial fluid pressure. Moreover, VEGF levels were found to be elevated in ocular fluid in patients with diseases characterized by hyperpermeability and neovascularization, specifically DR and retinal vein occlusion.\(^{15,16,17}\)

**The Pathogenesis of PDR**

Proliferative DR is characterized by neovascularization. To understand the role of VEGF in new vessel formation for PDR, remember that retinal tissue has one of the highest metabolic rates in the body, and oxygen demand in the retina is always near maximum—even under normal circumstances. Even mild vascular insufficiency can lead to ocular hypoxia and ischemia. Capillary occlusion, as seen in DR, can result in severe hypoxia, which is the main condition for initiating neovascularization in PDR.\(^8\) Hypoxia stimulates the production of VEGF mRNA.\(^9\) In several animal models of ischemia, VEGF expression is spatially and temporally correlated with neovascularization.\(^{16,18}\)

**The Pathogenesis of DME**

Two processes are involved in the pathogenesis of DME: malfunction of the blood-retina barrier and inflammation. Breakdown of the blood-retina barrier in patients with diabetes leads to the accumulation of plasma proteins and oncotically active fluid in the extracellular space, causing retinal swelling and neuronal disorganization. Thus, a combination of events determines whether the blood-retina barrier will remain intact: direct paracellular transport, alterations in endothelial intercellular junctions, and endothelial cell death. VEGF causes vascular hyperpermeability through multiple mechanisms, including opening interendothelial junctions and inducing fenestration (ie, cell junctions that are highly permeable to fluids and small solutes, which can thus facilitate bidirectional transport), increasing transcellular bulk flow, and inducing leukocyte-mediated vascular injury (VEGF is a potent chemoattractant for monocytes).\(^{19-21}\) VEGF also can induce phosphorylation of the tight junction proteins (ie, occludins and claudins, which limit flow between endothelial cells) and zonula occludens-1 (ZO-1), ZO-2, and ZO-3 (which are thought to organize tight junctions).\(^{21,22}\)

Inflammation is considered to be very important in the pathogenesis of ocular diabetic lesions and leukostasis (ie, abnormal leukocyte aggregation, in which activated leukocytes adhere to the retinal vascular endothelium), adhesion molecule activation, prostacyclin upregulation, and accumulation of macrophages. Adherent leukocytes also directly induce endothelial cell death in capillaries, causing vascular obstruction and leakage.\(^{23}\)

Ischemia also sets off a cascade of events through PKC activation that leads to leakage and exudation.\(^4\) VEGF promotes proliferation and migration of endothelial cells in addition to the elicitation of de novo vessel formation. VEGF also triggers the production of metalloproteinases required for the breakdown of basement membrane and the invasion of blood vessels into the surrounding stroma.\(^{24}\)

**The Etiologic Link Between Ocular and Systemic Diabetes Complications**

Prolonged hyperglycemia appears to be responsible for diabetes-related complications. It is now well established that diabetic neuropathy, nephropathy, and retinopathy are strongly correlated with metabolic control of the disease. Duration of diabetes is also clearly involved in the prevalence of microvascular complications, inflicting cumulative microvascular damage and escalating involvement of other risk factors. It is likely that diabetes-related complications share, at least in part, similar pathogenetic mechanisms in different cells and tissues.

The presence of DR is associated with renal damage, and DR and nephropathy are risk factors for development and progression of diabetic neuropathy. Elevated urinary albumin excretion rates have been found in 10% of patients with minimal DR who progressed to more advanced forms of retinopathy. In patients with type 1 diabetes, the presence of DR is predictive of microalbuminuria onset.\(^{25}\)

Diabetic neuropathy is caused by an alteration in nerve blood flow, although recent evidence points toward alterations in neural signaling.\(^{26}\) Hyperglycemia leads to nerve damage through the same mechanisms involved in DR, including activation of the polyol pathways, synthesis of AGEs, and PKC activation.\(^{27,28}\) Moreover, elevated serum AGE levels play a role in the progression of diabetic nephropathy, and AGEs have been found localized in nodular lesions in nephropathic kidneys, in which they impair protein assembly in vivo.\(^{29}\)
Hypertension is especially common in newly diagnosed type 2 diabetes and is often associated with obesity. Hypertension is an independent risk factor for the development and progression of DR and nephropathy, as shown by the United Kingdom Prospective Diabetes Study. In fact, there was a direct relationship between the risk of all diabetes complications and systolic blood pressure, such that each 10-mm Hg decrease in updated mean systolic blood pressure was associated with reductions in risk of 12% for any complication related to diabetes (95% confidence interval, 10%–14%; \( P < .0001 \)), 15% for deaths related to diabetes (12%–18%; \( P < .0001 \)), 11% for myocardial infarction (7%–14%; \( P < .0001 \)), and 13% for microvascular complications (10%–16%; \( P < .0001 \)). Importantly, the investigators also noted that no threshold of risk was observed for any endpoint—the greater the reductions in blood pressure, the better the outcomes for patients.

More recently, a study of 139 volunteers (normotensive, \( n = 74 \); hypertensive \( n = 47 \); and history of cerebrovascular event, \( n = 18 \)) found that an increased wall-to-lumen ratio of retinal arterioles was significantly higher in patients with a history of cerebrovascular events, compared with hypertensive and normotensive subjects, suggesting the use of retinal arterioles as possible tools to identify patients with increased risk of a cerebrovascular event. As the authors note, “retinal vessels can be regarded as a mirror of the cerebral vasculature.” In another small study, 19 patients with essential, mild hypertension were treated with losartan (an angiotensin receptor antagonist) or atenolol (a \( \beta \) blocker). After 1 year, losartan was able to significantly reduce the media:lumen ratio, with no change observed in those treated with atenolol, even though reductions in blood pressure were comparable in both groups. In addition, acetylcholine-induced endothelium-dependent relaxation was normalized in those receiving losartan, but not in those receiving atenolol. Sodium nitroprusside-induced endothelial relaxation was unchanged after both treatments. (Sodium nitroprusside acts directly on the vascular smooth muscles to cause relaxation and not on the endothelium. Thus, considering that the present study was conducted to test changes in endothelial dysfunction induced by different antihypertensive drugs acting on the endothelium, it was expected that the endothelium-independent relaxation was unchanged.)

### A NEW PROPOSED SEVERITY SCALE

The ETDRS proposed a classification system for DR, which is considered to be the gold standard. However, it uses a photographic grading system with several levels, making it difficult for the clinician to remember. In short, although it is reproducible and valid, it is impractical to use in everyday clinical practice. Wilkinson et al have proposed a very simple system for classification of and distinguishing nonproliferative and proliferative DR. The aim of this new system is to provide an accepted, standardized set of definitions describing the severity of DR and DME for clinical decision making, optimal communication among caregivers (especially between diabetologists and ophthalmologists), and research in different settings. It is based on the risk of disease progression, but it is not intended as a guide for treatment. It uses an evidence-based approach based on results from the ETDRS and Wisconsin Epidemiological Study of Diabetic Retinopathy results.

### Table 1. Proposed International Clinical DR Disease Severity Scale

<table>
<thead>
<tr>
<th>Proposed Disease Severity Level</th>
<th>Findings Observable with Dilated Ophthalmoscopy</th>
</tr>
</thead>
<tbody>
<tr>
<td>No apparent DR</td>
<td>No abnormalities</td>
</tr>
<tr>
<td>Mild nonproliferative DR</td>
<td>Microaneurysms only</td>
</tr>
<tr>
<td>Moderate nonproliferative DR</td>
<td>More than “mild” but less than “severe”</td>
</tr>
<tr>
<td>Severe nonproliferative DR</td>
<td>Any of the following:</td>
</tr>
<tr>
<td></td>
<td>• ≥20 intraretinal hemorrhages in ≥4 quadrants</td>
</tr>
<tr>
<td></td>
<td>• Definite venous beading in ≥2 quadrants</td>
</tr>
<tr>
<td></td>
<td>• Prominent IRMA in ≥1 quadrant and no neovascularization</td>
</tr>
<tr>
<td>Proliferative DR</td>
<td>≥1 of the following:</td>
</tr>
<tr>
<td></td>
<td>• Definite neovascularization</td>
</tr>
<tr>
<td></td>
<td>• Preretinal or vitreous hemorrhage</td>
</tr>
</tbody>
</table>

Wilkinson et al proposed a system for classification of and distinguishing nonproliferative and proliferative DR that is simpler for the practicing ophthalmologist than the ETDRS classification. The proposed classifications are strictly linked with the clinical evolution of the lesions; the risk of progression of DR increases with the different severity levels. However, this system is not meant as a guide for treatment.

DR = diabetic retinopathy; ETDRS = Early Treatment Diabetic Retinopathy Study; IRMA = intraretinal microvascular abnormalities.

Adapted with permission from Wilkinson et al. *DME = diabetic macular edema; ETDRS = Early Treatment Diabetic Retinopathy Study.*

**Proposed Disease Severity Scale**

<table>
<thead>
<tr>
<th>Proposed Disease Severity Level</th>
<th>Findings on Dilated Ophthalmoscopy</th>
</tr>
</thead>
<tbody>
<tr>
<td>DME absent</td>
<td>No retinal thickening or hard exudates present in posterior pole</td>
</tr>
<tr>
<td>DME present</td>
<td>Some retinal thickening or hard exudates present in posterior pole</td>
</tr>
</tbody>
</table>

**If DME is present, it can be categorized as follows:**
- **Mild DME**
  - Some retinal thickening or hard exudates in posterior pole but distant from the center of the macula
- **Moderate DME**
  - Retinal thickening or hard exudates approaching the center of the macula but not involving the center
- **Severe DME**
  - Retinal thickening or hard exudates involving the center of the macula

The proposed scales are shown in Tables 1 and 2. Importantly, the proposed classifications are strictly linked with the clinical evolution of the lesions; the risk of DR progression increases with the different severity levels. There are 3 levels with relatively low risk of progression to PDR (ie, no retinopathy, mild, and moderate nonproliferative retinopathy), and 2 levels with significant risk of visual loss (ie, severe nonproliferative retinopathy and PDR). At Level 1 (ie, no apparent DR), there is a 1% risk of progression to PDR after 4 years. However, at Level 4 (severe non-proliferative retinopathy and PDR), the risk of progression is 17% and 40% at 1 and 4 years, respectively.

**References**


**Conclusions**

Vascular endothelial growth factor is the most well-studied component of pathologic pathways involved in DR, and anti-VEGF therapies are currently being evaluated in clinical trials. However, even in successful trials, ocular neovascularization and tumor growth was slowed, but still progressed following administration of anti-VEGF drugs. Truly successful angiogenesis suppression (for treatment of not only ocular neovascularization but also DME) may require agents or therapies aimed at multiple molecular targets, reflecting the complexity of the angiogenic pathways.


