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# **Proliferative Diabetic Retinopathy: An Overview of Vitreous Immune and Biomarkers**

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Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/intechopen.74366>

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## **Abstract**

This chapter discusses about the effect of vitreous immune system and biomarkers on the progression of proliferative diabetic retinopathy. Immune system and biomarkers have been believed to have an important role in the progression of diabetic retinopathy (DR) severity. Hyperglycemic will influence immune cells resulting in chronic inflammation on the retina. This condition progressively disrupts the blood-retinal barrier in retina causing those inflammatory molecules and immune cells to transfer from circulation. The transfer of these molecules plays an important part in the progression of proliferative diabetic retinopathy. In addition, biomarkers are indicators for some complex processes happened in our body, and are measured to determine diagnosis and prognosis of some treatment. There are several biomarkers that have been identified in DR patients including biomarkers of oxidative stress, hypoxia-inducible factors, angiogenic factors, pro-inflammatory cytokines, chemokines, cell adhesion molecules, and soluble CD200. The value of these biomarkers will tell us their possible role in the progression of DR. By improving the knowledge of molecular pathway in DR pathophysiology, the advancement of selective therapy approaches could be discovered and the management of DR could be more efficient.

**Keywords:** biomarker, diabetic retinopathy, hyperglycemia, immune system, inflammation

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## **1. Introduction**

Diabetic retinopathy (DR) is the most common chronic microvascular complication of uncontrolled diabetes mellitus leading to preventable blindness. Diabetic retinopathy is often classified based on its severity into mild non-proliferative diabetic retinopathy (NPDR), moderate

NPDR, severe NPDR, and proliferative diabetic retinopathy (PDR) [1–3]. The major risk factors for developing DR are the duration of diabetes, hyperglycemia, hypertension, and dyslipidemia [4]. Glucose concentration increases in retinal cells leading to saccular capillary microaneurysms, pericyte deficient capillaries, and degenerate capillaries that decrease the retinal perfusion and contribute to the progression of DR [4]. Several types of evidence prove the benefits of tight glycemic and blood pressure control in decelerating the progression of DR. Nevertheless, the numbers of DR patients and the development of DR complications are still increasing, while therapeutic approaches are limited [1, 2].

For the last several decades, many studies have been performed in order to better understand DR progression from a molecular viewpoint. The biochemical mechanisms implicated in DR progression have been shown in various animal models and patients with diabetes [1]. It is believed that the involvement of hyperglycemia and hormonal factors in diabetic patients could disturb hemostasis in the retina and change the balance of some mediators including growth factors, cytokines, inflammatory, and adhesion molecules [5]. These changes result in altered capillary permeability, apoptosis of capillary cells, and angiogenesis, leading to DR complications [3]. With improved clarity of molecular pathways in DR pathophysiology, the advancement of selective therapeutic approaches could be discovered and the management of DR could be more effective [1, 5]. This chapter focuses on the inflammatory molecules and biomarkers involved in the pathophysiology of DR.

## **2. The immune system in proliferative diabetic retinopathy**

The immune system protects the body from both exogenous pathogens called pathogen-associated molecular patterns (PAMPs) and endogenous harmful molecules known as damage-associated molecular patterns (DAMPs). DAMPs include oxidized or glycated proteins, mislocated proteins/antigens, and intracellular contents released by necrotic cells. In normal conditions, the immune system regulates the inflammatory process and prevents uncontrolled inflammation that damages cells. In hyperglycemic conditions, the accumulation of DAMPs induces chronic inflammation in various tissues, which in turn manifests into the various complications of diabetes, including diabetic retinopathy [6].

The retina is one of few tissues in the human body that has immune privilege. It is protected from the attack of the systemic immune system by a series of complex defense mechanisms. This protection is afforded by a physical barrier formed between endothelial cells of retinal vasculature as the inner blood-retinal barrier (BRB) and retinal pigmented epithelial cells as the outer BRB. This barrier limits the movement of cells and molecules from the systemic circulation into the retinal parenchyma. The BRB also separates retinal antigens within the intraocular compartment, avoiding activation of T cells. This phenomenon is known as immunological ignorance. In addition, there is no lymphatic system in the retina. This inhibits systemic immune cells from detecting damage-associated molecular patterns in the retina thus preventing an overt systemic inflammatory response. Retinal cells (retinal neurons and RPE cells) express immune modulators that can suppress immune cells and complement system activation. The retina is protected by the local innate immune system (microglia, perivascular macrophages, and the complement system) whose activation is tightly controlled [6].

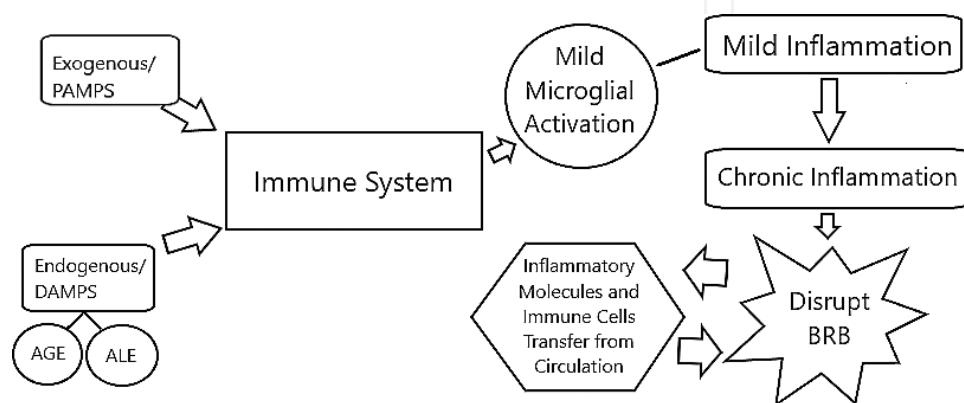
The immune system plays an important role in the progression of DR. Under hyperglycemic conditions, over activation of the innate immune system takes place, resulting in chronic inflammation of the retina. A study by Urbančič et al. showed the presence of T lymphocytes in the vitreous of patients with PDR. They found that the CD4/CD8 lymphocyte ratio in vitreous is higher compared to the blood ratio in these PDR patients, demonstrating the presence of a local inflammatory process [7]. Prolonged local inflammation in hyperglycemic conditions in the retina may develop into a chronic inflammatory response that is detrimental to the integrity of BRB [6, 8–10]. The destruction of the barrier shifts the retina from its “privileged state” when the BRB functions normally to “compromised state” when the BRB has broken down. Complement system activation also increases in diabetic conditions and this dysregulated activation is known to be involved in the degeneration of retinal vessels. Dysfunctional barriers permit inflammatory molecules and immune cells from systemic circulation to enter the retina and cause further deterioration of the tissue [6, 11]. Cytological examination of the vitreous samples from PDR patients were found to contain significant amounts of macrophages suggesting the infiltration of systemic immune cells into the retina [12, 13]. In addition, there was an increase in adhesion molecule expression and pro-inflammatory cytokine production, suggesting the role of defective neutrophil activity in the development of chronic inflammation in diabetic retinopathy [14, 15] (**Figure 1**).

### 3. Vitreous biomarkers in proliferative diabetic retinopathy

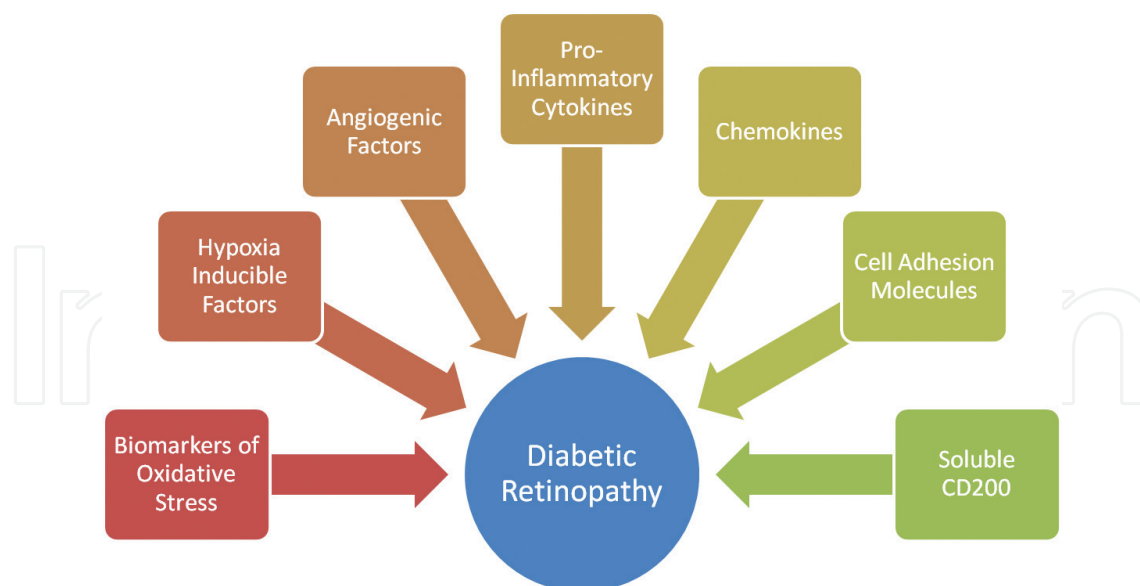
A biomarker is an objective measurement that is evaluated as an indicator for some complex processes happening in our body [16]. Biomarkers are usually measured to determine the diagnosis and prognosis of some treatments [17]. There are several biomarkers that can be found in diabetic retinopathy patients including biomarkers of oxidative stress, hypoxia-inducible factors, angiogenic factors, pro-inflammatory cytokines, chemokines, cell adhesion molecules, and CD200. The value of these biomarkers tells us their possible role in the progression of diabetic retinopathy [5, 6, 18–21] (**Figure 2**).

#### 3.1. Biomarkers of oxidative stress

The presence of oxidative stress biomarkers indicate an imbalance of reactive oxygen species (ROS) and the functional capabilities of cellular antioxidants [18, 22]. This imbalance can cause



**Figure 1.** Immune system role in progression of diabetic retinopathy.



**Figure 2.** Vitreous biomarkers involved in proliferative diabetic retinopathy.

cell instability and contribute to the development of many diseases, including diabetic retinopathy [18, 23]. Oxidative stress will remain high even after the patient reaches a normoglycemic state. This phenomenon is called “metabolic memory” and can lead to the accumulation of ROS in diabetic patients [24]. The biological markers of oxidative stress can include changes in molecules of the antioxidant system and molecules modified by ROS. Antioxidant enzymes like the superoxide dismutases are an example of changes in molecules of the antioxidant system, and malondialdehyde is the best known oxidative stress marker [18].

### 3.1.1. Superoxide dismutases

Superoxide dismutases (SODs) are a group of enzymes found in our cells, which function as major antioxidant defense systems against ROS in the body. SODs consist of three isoforms: the cytoplasmic Cu/ZnSOD (SOD1), the mitochondrial MnSOD (SOD2), and the extracellular Cu/ZnSOD (SOD3), all of which require catalytic metal (Cu or Mn) to activate. SOD activities will increase due to the presence of oxidative stress in the body. Vitreous SOD activity can also be used to measure oxidative stress levels inside the eye, allowing it to be a viable biomarker of oxidative stress in patients with PDR. Brzović-Šarić et al. state that PDR patients serum oxidative stress markers were higher than non-diabetic patients with an eye disorders (NDED) serum. Brzović-Šarić et al. found a mean activity level of SODs in the vitreous of male diabetic patients at  $30.5 \pm 2.5$  U/mL, and  $28.5 \pm 3.8$  U/mL in vitreous of female patients with diabetes [25]. Our previous study found a mean activity level of SODs in vitreous of patients with PDR at  $0.403 \pm 0.50$  U/mL [26].

### 3.1.2. Malondialdehyde

Malondialdehyde (MDA) is a highly reactive compound produced by lipid peroxidation of polyunsaturated lipid found in cell membranes. MDA exerts its oxidative stress effect inside

cells and forms molecules called advanced lipoxidation end-products (ALE). MDA levels in specific tissues can be measured to represent oxidative damage induced by physical or chemical oxidative stress in the corresponding tissues [24, 25, 27]. Brzović-Šarić et al. found a significant difference between vitreous MDA values in non-diabetic patients with an eye disorder and PDR patients [25]. On the other hand, several studies found an increase in MDA serum of diabetic patients compared to control patients, but there was no significant difference in MDA serum level between non-proliferative DR and proliferative DR patients [24, 27]. Our study found a mean activity level of MDA in the vitreous of patients with PDR at  $1.661 \pm 1.21$  nmol/mL [26]. Another study about oxidative stress levels with PDR by Mancino et al. found a mean activity level of MDA in vitreous of patients with PDR at  $520 \pm 210$  nmol/mL [24]. What causes these differences in vitreous MDA levels still needs to be explored.

### 3.2. Hypoxia-inducible factors

HIF-1 $\alpha$  is a DNA-binding protein complex that is continuously expressed and degraded by cells in the body. Under hypoxic conditions, the HIF-1 $\alpha$  degradation rate decreases, causing increased concentration of HIF-1 $\alpha$  which then translocates into the nucleus and dimerizes with HIF-1 $\beta$ . The HIF-1 complex then regulates the expression of genes responsible for the hypoxic response of the cell by binding into the hypoxia response element (HRE) [28]. The HIF-1 complex is known to cause angiogenic effects on these hypoxic tissues [29]. Previous studies by Arden et al. on patients with diabetic retinopathy shows that hypoxia is present in retinal tissues suffering from oxidative damage [30]. Accordingly, Wang and co-workers found increased levels of HIF-1 $\alpha$  protein in vitreous samples of PDR patients compared to levels in non-diabetic subjects [28]. Furthermore, the vitreous levels of vascular endothelial growth factor (VEGF) and HIF-1 $\alpha$  were highly correlated in PDR patients. Several studies demonstrated positive immunohistochemical staining for HIF-1 $\alpha$  and VEGF proteins in epiretinal neurovascular membranes. This evidence shows that HIF might play an important role in regulating the neovascularization of retina in PDR [31, 32].

### 3.3. Angiogenic factors

Angiogenesis is a complex multistep process that involves angiogenic factors and is induced by various cytokines and growth factors [33]. These factors have been suggested to be correlated with the development of diabetic retinopathy [5, 33–35]. These are also known to be hypoxia-responsive factors [5, 35]. Pro-angiogenic factors, like VEGF, angiopoietin, and erythropoietin are well-known factors contributing to neovascularization and whose levels increase in diabetic retinopathy patients [3, 5, 33–35]. Several therapies designed to target these factors have been proven effective in decreasing the progression of the disease [5].

#### 3.3.1. Vascular endothelial growth factor

Vascular endothelial growth factor (VEGF) is a signaling molecule that promotes development of new blood vessels. It is released by cells in response to hypoxic conditions. Abcouwer stated that VEGF increases vascular permeability by promoting the disassembly of junctions between endothelial cells. This leakage can cause diabetic macular edema

(DME) [5]. Several studies have shown marked increases of VEGF in vitreous and vitreous compared to plasma concentration in DME and PDR patients [19, 36–46]. Treatments that target VEGF have been proven highly effective in treating DR. VEGF antibodies, which were originally used for cancer treatments, such as bevacizumab and its correlate ranibizumab have been used effectively. These have also been tested in several small trials which showed improved vision in DR patients, demonstrating the involvement of VEGF in the pathophysiology of PDR [5, 36].

Brzović-Šarić also demonstrated a significant difference between vitreous VEGF values in non-diabetic patients with eye disorders and PDR patients [25]. Loukovaara et al. state that VEGF is a major factor in PDR development and found significant increases of VEGF levels in the vitreous of DR patients ( $465.1 \pm 1470.2$  pg/mL) compared to control patients ( $40.3 \pm 165.8$  pg/mL) [37]. Our study found a mean level of VEGF in vitreous of patients with PDR of  $0.356 \pm 0.60$  pg/mL [26]. Yoshimura et al. found that there was significantly elevated VEGF in PDR patients, but not in DME patients [47]. The increased levels of VEGF expression in patients with diabetic retinopathy was mainly produced by Muller glial cells. Experiments in diabetic mice, demonstrated that conditional knockout of VEGF in Muller cells effectively blocked the increase in retinal VEGF expression [48]. Lange and co-workers suggest that oxygen tension levels were positively correlated with vitreous VEGF levels, and oxygen tension levels at the posterior pole were increased in PDR patients [49]. The vitreous levels of VEGF will decrease in the most severe stage of PDR, when there is a transition from angiogenesis to fibrosis [50].

### 3.3.2. Angiopoietin

Angiopoietins are a group of proteins with the role of regulating vascular development and angiogenesis. Two types of angiopoietins, angiopoietin-1 and angiopoietin-2, contribute to the maintenance of retinal vasculature. The former exerts a stabilizing effect on vessels, organizing and limiting the angiogenesis response, while the latter exhibits angiogenic activity if VEGF is present, but promotes endothelial cell death and vascular regression in the absence of VEGF. The ratio between these two angiopoietins represents the inflammatory process in the cell. Fiedler et al. state that hypoxia/ischemia activates endothelial cells upregulating angiopoietin-2 thus lowering the angiopoietin-1/angiopoietin-2 ratio [37, 51]. A recent publication by Loukovaara et al. demonstrates significant correlation between intravitreal concentrations of Ang-2 with MMP-9, VEGF, EPO and TGF $\beta$ 1 levels in diabetic eyes undergoing vitrectomy, indicating its role in retinal tissue neovascularization in PDR patients. The study shows a slight increase in angiopoietin-1 from the control group ( $19.1 \pm 25.4$  pg/mL) to the study group ( $25.6 \pm 27.1$  pg/mL), and a great increase in angiopoietin-2 from the control group ( $43.0 \pm 60.9$  pg/mL) to the study group ( $317.1 \pm 419.1$  pg/mL), thus lowering the angiopoietin-1/angiopoietin-2 ratio in study group. The plasma value of angiopoietin-1 is similar in both groups, but the plasma value of angiopoietin-2 is increased from the control group ( $2623.4 \pm 2142.0$  pg/mL) compared to the study group ( $5690.4 \pm 8064.7$  pg/mL) [36, 37, 52]. Several studies state that angiopoietin-1 can be used for the prevention and treatment of diabetic retinopathy by its ability to suppress VEGF expression in diabetic retina [53].

### 3.3.3. Erythropoietin

Erythropoietin (EPO) is a glycoprotein cytokine that acts as a major regulator of erythropoiesis. Besides erythropoiesis, several studies state that erythropoietin has a neuroprotective and angiogenic effect in brain and retina. Production of EPO in serum and vitreous is mainly caused by hypoxia [54–57]. EPO is found in many organs, including kidney, liver, brain, and retina [55]. The angiogenic effect of EPO is a potential equivalent to VEGF, and has been suspected as an important factor in the angiogenesis of PDR [56]. Watanabe et al. showed that vitreous EPO levels of PDR patients are significantly higher (464.0 mIU/mL) compared to non-diabetic patients (36.5 mIU/mL). They also found that EPO levels are higher with active as compared to quiescent PDR [54]. These are consistent with Katsura et al., who also reported increases of vitreous EPO levels in PDR patients compared to controls [55]. Cristina et al. found that EPO levels in vitreous fluid are significantly higher (326 mU/mL) compared to serum EPO (11.2 mU/mL) in PDR patients [56]. This shows that intraocular production is responsible for the high concentration of erythropoietin found in the vitreous fluid of retinal degeneration patients [54, 56, 57]. Garci et al. found increased vitreous EPO concentrations in DME patients (430 mU/mL) compared to control patients (25 mU/mL) [57]. Treatment involving the erythropoietin blockade is likely to be beneficial, but may worsen the disease due to the decrease of its neuroprotective function [54].

### 3.3.4. Matrix metalloproteinases 9

Matrix metalloproteinases (MMPs) are a family of zinc ion-binding endopeptidases that degrade most of the extracellular matrix (ECM). MMPs regulate many cellular functions including apoptosis, wound healing, and angiogenesis. In angiogenesis, MMPs increase VEGF production and remove physical barriers to new vessel growth [58, 59]. MMPs are produced as a response to increased oxidative stress. Diabetic patients often have increased MMP, mainly MMP-9 and MMP-2 in the retina and vitreous. These are controlled by endogenous tissue inhibitors of metalloproteinases (TIMPs). TIMP-1 regulates MMP-9 and TIMP-2 regulates MMP-2 [59]. Several studies suggest that MMPs are responsible for many diabetic complications, including cardiomyopathy, nephropathy, and retinopathy. MMPs are suspected to facilitate apoptosis of retinal capillary cells during early stages leading to disruption of blood-retinal barrier integrity [58–60]. Kowluru et al. found an increase in MMP-9 and a decrease in TIMP-1 in the retina of DR patients [58]. Abu et al. found significant increases in vitreous zymography levels of MMP-9 in PDR patients ( $392.3 \pm 253.6$  scanning units) compared to non-diabetic control patients ( $168.2 \pm 65.0$  scanning units). However, the levels of vitreous MMP-2 in PDR patients ( $540.9 \pm 185.6$  scanning units) did not differ significantly from non-diabetic control patients ( $505.4 \pm 216.1$  scanning units) [60]. Inhibitors of MMPs have been used to treat several diseases, however, there have been no studies using these inhibitors to treat DR patients [59].

### 3.3.5. Transforming growth factor $\beta$

Transforming growth factor  $\beta$  (TGF- $\beta$ ) is a polypeptide responsible for controlling cell proliferation and differentiation. It is usually secreted in a latent phase and must be transformed



to become a mature active form. In the human eye, there are three known TGF- $\beta$  isoforms (TGF- $\beta_1$ , TGF- $\beta_2$ , and TGF- $\beta_3$ ), where the posterior segment of the eye mainly contains TGF- $\beta_2$  as the dominant form [61–63]. Hirase et al. found an increase in total vitreous TGF- $\beta_2$  levels in PDR patients ( $2634 \pm 1652$  pg/mL) compared to control patients ( $1305 \pm 972$  pg/mL) [61]. This result is also consistent with a McAuley et al. study about vitreous biomarkers in diabetic retinopathy [62]. The mature active form of TGF- $\beta_2$  levels are also increased in PDR patients. This increase correlates with the disease severity, suggesting that TGF- $\beta_2$  angiogenesis properties play a role in the progression of PDR [61].

### 3.4. Pro-inflammatory cytokines

Pro-inflammatory cytokines are usually secreted by inflammatory cells in response to hypoxia or hyperglycemia [64]. Well-known pro-inflammatory cytokines, such as tumor necrosis factor, interleukin, interferon, and receptor tyrosine kinase are found to be elevated in the vitreous of diabetic retinopathy patients, suggesting their important role in the pathogenesis of this disease [5, 64, 65]. Cytokines can induce the progression of diabetic retinopathy directly and indirectly. Direct mechanisms include the direct engagement with target cells to induce neovascularization [64]. While indirect mechanisms induce leukocytes and endothelial cells to produce pro-angiogenic mediators, which in turn induce neovascularization [64, 65]. Therapy targeting these cytokines may be beneficial, but we need better understanding about the cytokine roles to do so [5].

#### 3.4.1. Tumor necrosis factor- $\alpha$

Tumor necrosis factors- $\alpha$  (TNF- $\alpha$ ), a pro-inflammatory cytokine, is primarily synthesized by macrophages and T cells. Its expression is regulated by NF- $\kappa$ B and it has been associated with the pathogenesis of several chronic inflammatory diseases including type 2 diabetes. Its function is primarily as an immune-modulator and it also plays a role in neovascularization and fibroplasia [3]. Costagliola et al. suggest that TNF- $\alpha$  is a potent mediator of leukostasis and contributes to blood-retinal barrier breakdown [3, 66]. TNF- $\alpha$  concentration is found elevated in the vitreous of PDR patients and the vitreous/serum ratio of TNF- $\alpha$  is also found higher compared to non-diabetic patients. Costagliola et al. found that TNF- $\alpha$  levels were lower in controls (1.9 pg/mL) than the PDR group (13.5 pg/mL) and increased with the severity of the disease [3, 66]. TNF- $\alpha$  has a short half-life ( $\sim 4$  min), making its analyzation prone to producing false negative results. Soluble TNF- $\alpha$  receptors (sTNF- $\alpha$ -Rs) have a longer half-life, making it a more reliable marker of the activation of TNF- $\alpha$  system [29, 31, 67–71].

#### 3.4.2. Interleukin

Several studies have shown that there is involvement of interleukins in the development of PDR. The most common interleukins found in DR patients are IL-6 and IL-8, where their concentrations were found increased in the vitreous of patients with PDR and prolonged hyperglycemia [3, 42, 47, 72–82]. Their role in the pathogenesis of PDR is still under investigation but evidence suggests the possibility of a rather direct contribution. IL-6 controls immune cells responses by shifting T-helper cell populations, inhibiting the production of Th1 cells, promoting the differentiation of Th2 and Th17 cells, and infiltration of monocytes and T cells [9, 10, 83].

*In vitro* study of IL-6 reports its ability to increase endothelial cell and vascular cell permeability by rearranging actin filaments and by changing the shape of endothelial cells [3, 65]. Several studies state that IL-6 also plays an important role in angiogenesis by activating VEGF, and regulating expression of metalloproteinases [3, 64]. IL-8 is known to be a potent angiogenic factor and also a potent chemoattractant and activator of neutrophils and T lymphocytes [64, 84, 85]. Increase of IL-8 concentrations in PDR patients, suggest that they are upregulated in response to oxygen stress and contribute to triggering inflammatory reactions. Study by Takahashi et al. shows that there is a significant increase in IL-6 and IL-8 values in PDR patients (918.0 and 2168.0 ng/mL) compared to control patient (517.0 and 343.0 ng/mL) [85]. Elner et al. also found increased levels of IL-8 in active PDR patients ( $24.7 \pm 4.5$  ng/mL) compared to control patients ( $7.5 \pm 2.3$  ng/mL), however inactive PDR patients ( $11.6 \pm 5.2$  ng/mL) did not differ significantly from controls [79]. It is most likely that VEGF expression causes an increase of IL-8 [86]. On the other hand, IL-10 concentration is not increased in the vitreous of patients with PDR. IL-10 is another important immunoregulatory cytokine that is induced by cell hypoxia. IL-10 activates nitric oxide and increases vascular permeability during the development of PDR [3, 65, 84, 85].

#### 3.4.3. Monokine induced by interferon- $\gamma$

Monokine induced by interferon- $\gamma$  (Mig) attracts activated T cells and has potent angiostatic activity. Several studies suggest that Mig correlates with VEGF and contributes to the progression of neovascularization in DR patients. The main function of Mig in the progression of DR might be related to its leukostasis function [88, 89]. Wakabayashi et al. found significant increases in vitreous concentration of Mig in active (148 pg/mL) and inactive (82.3 pg/mL) DR patients compared with non-diabetic patients who had macular disease (21 pg/mL). However, there was no significant difference in serum Mig concentration between DR patients (85.9 pg/mL) and control subjects (70.4 pg/mL) [87]. Takeuchi et al. also found an increase in Mig vitreous concentration in PDR patients compared to epiretinal membrane patients, idiopathic macular hole patients, and uveitis patients [88].

#### 3.4.4. Receptor tyrosine kinase

Receptor tyrosine kinase (c-kit) is expressed by bone marrow and involved in intracellular signaling. It plays an important role in cell proliferation, cell adhesion, cell survival, and neovascularization [89]. Several studies have shown that C-kit plays an important role in the angiogenic process of PDR. C-kit has a soluble form called s-kit that can be generated by proteolytic cleavage [90]. Abu et al. found an increase of c-kit expression in membranes from patients with active neovascularization ( $697.4 \pm 1528.1$  pg/mL) compared to patients with inactive PDR ( $205.3 \pm 106.4$  pg/mL) and control patients ( $87.5 \pm 91.5$  pg/mL). This demonstrates that an increase of c-kit expression is correlated to the progression of PDR [90]. However, Lee et al. found a slight decrease of c-kit values in the PDR group compared to NPDR group [91].

### 3.5. Chemokine

Chemokines are low molecular weight proteins that have many functions, including enhanced immune responses, regulation of homeostasis, and controlling angiogenesis [20, 92, 93].

Chemokines are often referred to as secondary pro-inflammatory mediators, whose activation is induced by pro-inflammatory cytokines or primary pro-inflammatory mediators. Chemokines induce a specific leukocyte type and can bind to chemokine-receptors on target cells [20, 92]. Chemokines are usually categorized into two groups, the CXC group is chemotactic for neutrophils and the CC group is chemotactic for monocytes and lymphocytes [20, 92]. Several studies show an increase of chemokines in vitreous of PDR patients, suggesting that they have roles in mediating angiogenesis and fibrosis in PDR patients [20, 93, 94]. Struyf et al. stated that chemokines have different roles based on disease progression. In the early phase, chemokines can induce leukocyte attraction and in late phase, they can induce neovascularization [93]. Das et al. introduced a new therapy targeting chemokines in patients with DME [94].

### 3.5.1. Monocyte chemotactic protein-1

Monocyte chemotactic protein-1 (MCP-1) is a member of the chemokine group which is responsible for regulating migration and infiltration of monocyte/macrophages to the site of inflammation, making MCP-1 a pro-inflammatory cytokine that plays a central role in CNS inflammation [2]. Hyun et al. stated that MCP-1 is a major cause of vascular complications in diabetes [95]. It is also a potent inducer of angiogenesis and fibrosis. MCP-1 levels were found elevated in the vitreous of diabetic patients and their levels are higher than serum [2, 97]. Ning et al. stated that advanced glycation end product (AGE) stimulation activates retinal neurons to release MCP-1 activating retinal microglial cells. Their study also shows a progressive increase of MCP-1 along with the progression of disease, indicating it may be an important link in diabetic retinopathy pathogenesis [2, 96]. Hyperglycemia also has been shown to increase MCP-1 expression from retinal vascular endothelial cells, RPE cells, and Muller glial cells [2, 97]. Reddy et al. demonstrated significantly higher levels of MCP-1 in PDR patients compared to normal glucose tolerance (NGT) patients. MCP-1 is also steadily increased along with the progression of PDR [97].

### 3.5.2. Interferon gamma-induced protein-10

Interferon gamma-induced protein-10 (IP-10), also known as CXCL10, is one of the CXC chemokine members. CXC chemokine has unique properties in which it can act as either an angiogenic or angiostatic factor, depending on the protein configuration of the molecule. IP-10 is inducible directly or through activation of IFN- $\gamma$ , TNF- $\alpha$ , NFkB, viruses, or microbial products. Boulday et al. reported that VEGF induced the expression of IP-10 [1]. IP-10 binds CXCR3 receptors inducing apoptosis, angiostasis, and chemotaxis. It has been suggested that IP-10 is associated with inflammatory diseases including immune dysfunction and infectious disease. This protein has also been proposed to be involved in the pathophysiology of diabetic retinopathy, especially in the development of neovascularization. Elner et al. found a significant increase in the level of IP-10 in patients with PDR compared to the patients with non-diabetic eye diseases (NDED;  $11.7 \pm 1.1$  ng/mL and  $4.6 \pm 0.9$  ng/mL;  $p < 0.001$ , CI 95%). They also assumed that pan-retinal laser photocoagulation (PRP) might influence elevated IP-10 levels. The exact mechanism of the PRP-induced IP-10 involution of PDR remains to be elucidated [79].

### 3.5.3. Stromal cell-derived factor-1

Stromal cell-derived factor-1 (SDF-1) is a chemokine with a major role in the ischemic damage repair process. It recruits endothelial progenitor cells (EPCs) from the bone marrow to the site of repair and upregulates expression of VEGF, increasing the angiogenic process. This pro-angiogenic factor is categorized as being hypoxia-responsive and is found to be upregulated in PDR [98–100]. Chen et al. found that vitreous concentrations of SDF-1 and VEGF are correlated in eyes with PDR. They also found that vitreous levels of SDF-1 are significantly higher in PDR patients ( $306.37 \pm 134.25$  pg/mL) than in patients with idiopathic macular hole ( $86.91 \pm 55.05$  pg/mL) [101]. Butler et al. demonstrate an increase of SDF-1 concentration in the vitreous of patients with PDR and this increase correlates directly with disease severity. They also demonstrated that intravitreal injection of triamcinolone dramatically decreased the concentration of vitreal VEGF and SDF-1, suggesting it as another possible treatment for PDR [98].

### 3.5.4. High-mobility group box-1

High-mobility group box-1 (HMGB1) is a nonhistone DNA-binding protein that facilitates transcription. HMGB1 can be released into the extracellular space by active secretion from certain cells such as activated monocytes and macrophages, mature dendritic cells, natural killer cells, and endothelial cells. Necrotic cell death can also cause passive leakage of HMGB1 from the nucleus as the protein is no longer bound to DNA. HMGB1 can bind to the receptor for advanced glycation end products (RAGE) and toll-like receptor 2 (TLR-2), where it acts as a pro-inflammatory cytokine, activating NF- $\kappa$ B resulting in the overexpression of other pro-inflammatory molecules such as TNF- $\alpha$ , MCP-1, and ICAM-1 [41, 102, 103]. El-Asrar et al. demonstrated a significant correlation between neovascularization levels in epiretinal membranes of patients with PDR and the expression of HMGB1 and RAGE [41]. Yao Yu et al. also found an increase of HMGB1 concentration in the vitreous of PDR patients. This increase in vitreous happens in the later phases of DR, and differs from other inflammatory cytokines. They also found increases of RAGE protein and decreases of TLR-2 protein in DR rats, suggesting that the involvement of HMGB-1 is mainly through its binding with RAGE [102].

## 3.6. Cell adhesion molecules

Adhesion molecules have many roles in our body, including embryology, immunology, and malignancy [21]. Several studies show increases of these molecules in PDR patients, suggesting that cell to cell interaction plays a major role in the development of PDR [79, 104–106]. These molecules regulate lymphocyte recruitment to vascular endothelium. Well-known adhesion molecules found in PDR patients are intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecules-1 (VCAM-1), which are required for initiation of adhesion-dependent immune response [21, 106].

### 3.6.1. Intercellular adhesion molecule-1

Intercellular adhesion molecule-1 (ICAM-1), also known as CD54, is a cell surface glycoprotein encoded by the ICAM1 gene. ICAM-1 is usually expressed on the surface of endothelial

cells and cells of the immune system. It works as a cell adhesion molecule that recruits nearby circulating leukocytes to the inflamed location. In PDR patients, ICAM-1 is suspected as one of the deteriorating factors, promoting leukostasis and inflammation on nearby retinal tissue. Several experiments show leukostasis as a possible mechanism in diabetic retinal vasculature injury. Cells which are attached, mainly granulocytes and monocytes cause microvascular occlusion and capillary injury [106]. Leukostasis in DR is mainly caused by endothelial activation and increased surface expression of intercellular adhesion molecules (ICAM-1) [107]. Hillier et al. stated that increases in ICAM-1 correlate with the severity of DR in patients [108]. Our study on ICAM-1 showed an increase of ICAM-1 expression in PDR patients with more than 10 years of diabetes history [103]. Yan et al. in their study about the effects of intravitreal ranibizumab injection on ICAM-1 levels in PDR patients, demonstrated a decrease of ICAM-1 levels a week after intravitreal injection [109].

### 3.6.2. *Vascular cell adhesion molecule-1*

Vascular cell adhesion molecule-1 (VCAM-1) is an immunoglobulin supergene family of cellular adhesion molecules that are involved in the transmigration of monocytes, eosinophils, and lymphocytes [105, 110–112]. Oxidative stress, VEGF, and hypercholesterolemia increase the expression of VCAM-1 in the brain and retina [111, 113, 114]. It is released by endothelial cells and is present as an early feature of inflammatory disease [111, 113]. Several studies state that VCAM-1 promotes angiogenesis in PDR patients [105, 112–114]. Burgos et al. demonstrated increases in vitreous concentration of VCAM-1 in PDR patients (26 ng/mL) compared to non-diabetic patients in whom a vitrectomy was performed (22 ng/mL) [104]. These results are also consistent with Mroczek et al. in their study about the influence of glucose control on the activation of the intraocular molecular system [114]. There are also reports of increase VCAM-1 concentration in the retinal vessels and serum of PDR patients [111, 112].

### 3.7. Soluble CD200

CD200 is a novel immunosuppressive molecule found in neuronal cells. CD200 exists in a cell membrane-bound form and a soluble form. It exerts inhibitory effects on microglia/macrophages via interaction with the CD200 receptor (CD200R) [115]. DR-related neuronal degeneration also reduces CD200 concentration and further induces microglial activation [6]. Recent study on CD200 revealed that levels of sCD200 in vitreous of patients with PDR are significantly higher compared to that in the vitreous of patients without PDR. Xu et al. showed increases in mean sCD200 levels in the PDR group ( $182 \pm 17.63$  pg/mL) compared to non-diabetic patients with other conditions who requires pars plana vitrectomy ( $56.86 \pm 6.573$  pg/mL). This study also showed that vitreous levels of sCD200 are higher in PDR patients with DME ( $266.9 \pm 28.82$  pg/mL) or traction retinal detachment (TRD) ( $256.9 \pm 34.50$  pg/mL) compared to PDR patients without DME ( $136.9 \pm 15.13$  pg/mL) or TRD ( $146.9 \pm 15.97$  pg/mL). sCD200 level increases also have significant statistical correlations with the increase of several angiogenic and inflammatory molecules such as VEGF, IL-6, IL-8 and IL-10 [115].

## Acknowledgements

This article's publication is partially supported by the United States Agency for International Development (USAID) through the Sustainable Higher Education Research Alliance (SHERA) Program for Universitas Indonesia's Scientific Modeling, Application, Research and Training for City-centered Innovation and Technology (SMART CITY) Project, Grant #AID-497-A-1600004, Sub Grant #IIE-00000078-UI-1.

## Conflict of interest

There are no conflicts of interest in this chapter.

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